

Q2 11/4/57

National Institute
of
Allergy and Infectious
Diseases

Annual Report of Intramural Activities

October 1, 1985 - September 30, 1986

U.S. Department of Health and Human Services
Public Health Service
National Institutes of Health

National Institute
of
Allergy and Infectious
Diseases (U.S.)

Annual Report of Intramural Activities

October 1, 1985 - September 30, 1986

U.S. Department of Health and Human Services
Public Health Service
National Institutes of Health

For Administrative Use

RC

583

N277

1986

TABLE OF CONTENTS

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

1986 ANNUAL REPORT OF INTRAMURAL ACTIVITIES

INTRAMURAL RESEARCH PROGRAM

OFFICE OF THE SCIENTIFIC DIRECTOR.....	1-1
LABORATORY OF BIOLOGY OF VIRUSES.....	2-1
LABORATORY OF CLINICAL INVESTIGATION.....	3-1
LABORATORY OF IMMUNOGENETICS.....	4-1
LABORATORY OF IMMUNOLOGY.....	5-1
LABORATORY OF IMMUNOPATHOLOGY.....	6-1
LABORATORY OF IMMUNOREGULATION.....	7-1
LABORATORY OF INFECTIOUS DISEASES.....	8-1
LABORATORY OF MICROBIAL IMMUNITY.....	9-1
LABORATORY OF MOLECULAR MICROBIOLOGY.....	10-1
LABORATORY OF PARASITIC DISEASES.....	11-1
LABORATORY OF VIRAL DISEASES.....	12-1
ROCKY MOUNTAIN LABORATORIES	
LABORATORY OF MICROBIAL STRUCTURE AND FUNCTION.....	13-1
LABORATORY OF PATHOBIOLOGY.....	14-1
LABORATORY OF PERSISTENT VIRAL DISEASES.....	15-1
OPERATIONS BRANCH.....	16-1



NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

1986 ANNUAL REPORT REPORT PROJECT NUMBER LISTING*

Z01 AI

00013-23 LIP
00020-11 LVD
00027-19 LMM
00030-18 LI
00035-11 LI
00036-21 LI
00043-21 LCI
00045-18 LCI
00047-17 LCI
00048-16 LCI
00057-13 LCI
00058-12 LCI
00065-13 LMSF
00071-15 LPB
00072-15 LPVD
00074-14 LPVD
00082-25 LPB
00085-09 LPVD
00086-09 LPVD
00094-27 LPD
00097-28 LPD
00098-30 LPD
00099-16 LPD
00102-12 LPD
00103-19 LPD
00108-15 LPD
00123-20 LVD
00126-13 LVD
00131-19 LMI
00134-24 LMI
00135-12 LIP
00136-14 LMI
00138-12 LIP
00143-17 LMI
00144-22 LMI
00145-19 LMI
00147-11 LI
00148-11 LI
00153-09 LMI
00154-11 LCI
00155-11 LCI
00161-09 LPD
00162-10 LPD
00166-09 LIG
00168-09 LIG
00169-09 LIG

Z01 AI

00170-09 LIG
00171-09 LIG
00172-08 OSD
00173-09 LIG
00180-08 LIG
00182-08 OSD
00183-08 OSD
00186-13 LI
00189-07 LCI
00190-08 LMM
00192-08 LCI
00193-07 LMSF
00197-07 LPD
00199-07 LPVD
00201-07 LPB
00203-07 LI
00205-06 LIP
00208-06 LPD
00210-06 LIR
00212-06 LIR
00213-06 LIR
00216-06 LMSF
00218-05 LMM
00219-05 LMM
00222-05 LMM
00223-05 LI
00224-05 LI
00226-05 LI
00231-05 LPB
00234-05 LMSF
00235-05 LMSF
00240-05 LPD
00241-05 LPD
00242-05 LPD
00244-05 LPD
00246-04 LPD
00248-05 LPD
00249-05 LCI
00250-05 LCI
00251-05 LPD
00253-05 LPD
00255-05 LPD
00256-05 LPD
00257-05 LPD
00260-05 LPVD
00262-05 LPVD

*Does not include terminated or inactive projects.

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

1986 ANNUAL REPORT REPORT PROJECT NUMBER LISTING

Z01 AI

00263-05 LPVD
00264-05 LPVD
00265-05 LPVD
00266-05 LPVD
00269-05 LCI
00270-05 LCI
00271-05 LCI
00272-05 LCI
00275-05 LCI
00276-05 LCI
00277-05 LCI
00278-05 LCI
00279-05 LCI
00281-05 LMM
00284-05 LIP
00286-05 LIP
00290-05 LBV
00292-05 LBV
00294-05 LBV
00295-05 LBV
00296-05 LBV
00297-05 LBV
00298-05 LVD
00300-05 LMM
00301-05 LMM
00304-05 LMM
00306-05 LVD
00307-05 LVD
00308-05 LID
00309-05 LID
00311-05 LID
00312-05 LID
00314-05 LID
00316-05 LID
00317-05 LID
00319-05 LID
00321-05 LID
00323-05 LID
00324-05 LID
00325-05 LID
00326-05 LID
00327-05 LID
00333-05 LID
00335-05 LID
00338-05 LID
00339-05 LID

Z01 AI

00340-05 LID
00341-05 LID
00342-05 LID
00343-05 LID
00344-05 LID
00345-05 LID
00346-05 LID
00347-04 LPD
00348-04 LPD
00349-04 LI
00350-04 LPD
00351-04 LPD
00352-04 LIG
00353-04 LMM
00354-04 LCI
00356-04 LCI
00357-04 LCI
00358-04 LIR
00361-04 LIR
00366-04 LID
00368-04 LID
00370-04 LID
00372-04 LID
00386-02 LPVD
00388-03 LMM
00389-03 LIG
00390-03 LIR
00391-03 LVD
00392-03 LVD
00393-03 LVD
00394-03 LI
00395-03 LMM
00396-03 LCI
00397-03 LCI
00398-03 LCI
00402-03 LPB
00403-03 LI
00404-03 LID
00405-03 LID
00406-03 LID
00407-03 LID
00408-03 LID
00412-03 LMSF
00413-03 LMSF
00415-03 LMM
00416-03 LVD

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

1986 ANNUAL REPORT REPORT PROJECT NUMBER LISTING

Z01 AI

00417-03 OSD
00418-03 LPVD
00423-03 LMI
00425-02 LI
00427-02 LI
00428-02 LCI
00429-02 LCI
00430-02 LCI
00431-02 LIR
00432-02 LCI
00433-02 LMM
00434-02 LMM
00437-02 LMM
00438-02 LMM
00439-02 LPD
00440-02 LID
00441-02 LMSF
00442-02 LMSF
00443-02 LVD
00444-02 LVD
00445-02 LVD
00446-02 LBV
00447-02 LCI
00450-02 LID

Z01 AI

00451-02 LID
00457-02 LID
00458-02 LID
00459-02 LID
00462-02 LID
00464-01 LMM
00465-01 LIP
00466-01 LMM
00467-01 LMM
00468-01 LPVD
00469-01 LCI
00470-01 LCI
00473-01 LID
00474-01 LID
00475-01 LID
00476-01 LID
00477-01 LID
00478-01 LID
00479-01 LID
00480-01 LPB
00481-01 LCI
00482-01 LCI
00483-01 LPD





OFFICE OF THE SCIENTIFIC DIRECTOR, NIAID
1986 Annual Report
Table of Contents

<u>Z01-AI</u> <u>Project Number</u>		<u>Page</u>
Summary of Program		1-4
00417-03	Systematics and Vector Relationships of Ticks (Ixodoidea)--Keirans	1-5
00172-08	Structure and Synthesis of Peptides-- Maloy	1-6
00383-03	Acquisition of Specimens from Cases of Acquired Immune Deficiency Syndrome-- Salzman	1-7
00421-02	Analysis of Specimens Collected from Populations at Risks of AIDS--Waxdal	1-8
00228-04	Flow Cytometric Analysis of Cell Membrane Antigens and Receptors--Waxdal	1-9
00182-08	Biochemical & Genetic Mechanisms of Obligate Intracellular Parasitism-- Williams	1-10
00183-08	Immunologic Properties of <u>Coxiella</u> <u>Burnetii</u> (Q Fever) Vaccine--Williams	1-11



Summary of Program
Laboratory and Clinical Research, NIAID
October 1, 1985 - September 30, 1986

The Intramural Research Program (IRP) of the National Institute of Allergy and Infectious Diseases consists of 14 Laboratories. The individual research summaries describing the research in these laboratories are contained in the following pages. Eleven of the laboratories are located at the NIH campus in Bethesda, MD. Several Sections from the Bethesda laboratories as well as contract support services are at NIAID facilities at the Frederick Cancer Research Facility, Frederick, MD. The remaining three laboratories are located at the Rocky Mountain Laboratories (RML) in Hamilton, Montana. The Office of the Director, IRP (ODIRP), is responsible for the administrative management of the entire Intramural Research Program.

Effective in February, 1985, the Director, IRP, Kenneth Sell, M.D., Ph.D., left NIAID. The Acting Director after Dr. Sell's departure was Gordon Wallace, D.V.M., who served in this capacity until the appointment of the new Director, IRP, John I. Gallin, M.D., in November, 1985. Dr. Wallace retired from the Public Health Service in July, 1986.

Over the past year the organization of the ODIRP has changed to include an Administrative Management Branch and an Animal Care Branch. NIAID Technical Services have been consolidated under the ODIRP. NIAID Cell Sorters are now overseen by Dr. Thomas Chused and will be localized to a central facility. The Peptide Synthesis Facilities are now jointly supported by the IRPs of the NIAID and the National Institute of Dental Research. Other administrative changes introduced into the NIAID-IRP this year include a new approach for allocating the IRP budget. Much of the budget has been decentralized from the ODIRP to individual laboratories, giving more responsibility (and flexibility) to Laboratory Chiefs. An automated Data Processing Program has been initiated that will result in a local area network linking personal computers throughout the IRP and the entire Institute. A liquid nitrogen freezer repository that can house up to a maximum of 250,000 specimens has been opened at the NIAID Frederick facility and this will provide, at minimal cost, added freezer storage for NIAID scientists.

In an effort to more effectively utilize the resources at the NIAID-RML facility, the animal production program has been expanded to supply selected animals to the Bethesda campus at considerable cost savings. Several other cost-saving measures were undertaken. It was decided that previous plans to renovate the old Jefferson School adjacent to the RML laboratories for use as a conference center would not be cost efficient. Therefore, these plans were canceled. In the face of increasing budget cuts, it was decided that NIAID-IRP support for the Smithsonian Tick Collection (moved to the Smithsonian from RML in 1983) could no longer be continued. Therefore, all NIAID support for the tick collection will be phased out over a two-year period, providing ample time for the Smithsonian to get alternative funding.

NIAID animal facilities at RML and Frederick meet AAALAC standards and plans have been submitted to renovate existing facilities in Bethesda to assure that all NIAID animal facilities meet AAALAC standards.

NIAID-IRP has made a major effort in AIDS research during the past year. Much has been learned about the molecular biology of the virus. The work of the Laboratory of Molecular Microbiology has resulted in sensitive methods for titration of virus preparations and for monitoring the biological activity of viral DNA following infection of cells. Various cell systems have been identified that support the growth of the virus, and studies to develop immunogens that can be used for vaccines are underway. Clinical studies by the Laboratory of Immunoregulation have resulted in significant advances in understanding the immunopathogenesis of AIDS and development of therapeutic strategies. It has been shown that AIDS patients who develop antibody responses to gag proteins of the AIDS virus have a lower conversion rate to AIDS than do those patients who do not develop antibody to gag or who lose their response to gag. A number of antiretroviral agents are being studied although to date none have been shown to be effective. Recombinant interleukin-2 shows promise as a immune potentiator in AIDS and studies utilizing interleukin-2 with an effective antiviral agent will be undertaken as soon as an effective antiviral agent is discovered. Reconstitution of immune function was successful in one of three patients receiving a bone marrow transplant in conjunction with syngeneic lymphocyte transfusions.

In order to help meet the national AIDS urgency, this year the NIAID-IRP has initiated a vaccine development program with the purpose of supporting existing NIAID-IRP programs that are developing potential immunogens against the AIDS virus (HTLV-III), new vectors for vaccine delivery, and new antiviral agents. To launch this program two protein chemists and support staff have been hired by the contract mechanism and will be temporarily located adjacent to existing NIAID facilities in Frederick, MD. The permanent location of this AIDS vaccine development support program is expected to be building 468 in Frederick, MD, which we hope will be renovated in early FY'87.

Vaccine development against many microorganisms causing disease is a major focus of the NIAID-IRP Program. Studies by NIAID RML scientists have focused on developing a "clean" vaccine against Bordetella pertussis, the etiologic agent of whooping cough. Molecular cloning and expression of genes relevant to the toxic components of B. pertussis have been pursued and detailed information regarding cloning and sequencing of pertussis toxin genes have been published. The toxic and immunogenic domains of the pertussis toxin are being defined and studies, in collaboration with industry, are underway to develop an immunogen that will be used as a vaccine.

Studies of Chlamydia trachomatis, the most common sexually transmitted bacterial pathogen in the United States with 10 million cases annually, has resulted in a rapid screening test which should result in more rapid initiation of therapy. NIAID-IRP scientists have developed monoclonal antibodies to an epitope on the major outer membrane protein of chlamydia that protects mice from infection. This epitope is being investigated as a candidate immunogen for a vaccine in humans. Similar studies have been performed with Rickettsiae rickettsii, the etiologic agent of Rocky Mountain Spotted Fever. A 155-kd protein from R. rickettsii has been molecularly cloned into E. coli and the E.

coli has been used as a vaccine which is capable of eliciting protection for mice and guinea pigs. Preparations are now being made for a potential human vaccine.

In other studies of vaccine development significant progress was made by the Laboratory of Infectious Diseases to our understanding of the genome organization and function of various gene products of dengue virus, respiratory syncytial virus, parainfluenza type 3 virus, rotavirus and hepatitis A virus. Furthermore, a new strategy for immunization against respiratory syncytial virus was developed using vaccinia virus recombinants, a technique worked out by the Laboratory of Viral Diseases. Initial studies of these recombinants in experimental animals were highly successful. In other studies of infant diarrhea caused by rotavirus, clinical evaluation of the rhesus rotavirus candidate live vaccine strain provides a basis for optimism.

Clinical trials by the Laboratory of Parasitic Diseases towards development of a vaccine against malaria indicates that immune response to recombinant or synthetic malarial antigens may signal the need for more basic work. Phase I trials in humans of the recombinant anti-sporozoite malarial vaccine gave poor antibody response and no booster effect. Therefore, the phase II trials for efficacy have been postponed until (or unless) better immune responses can be obtained. In other studies the LPD has made progress defining protective immunity in leishmanial infections. Studies of Schistosomiasis have resulted in the cloning and sequencing of the protective schistosome antigens. This is a major advance towards development of a vaccine against Schistosomiasis.

Studies by Laboratory of Clinical Investigation investigators have demonstrated that acyclovir is an effective agent for diminishing recurrence of genital herpes virus infection. In addition, a double-blind clinical trial evaluating effects of acyclovir on chronic mononucleosis is underway.

Studies of scrapie slow virus infection by the Laboratory of Persistent Viral Infections (RML) have resulted in delineation of the complete cDNA sequence of mouse scrapie-associated prion proteins. An important advance in studying Scrapie disease is that the scrapie agent has been cultivated in vitro in mouse neuroblastoma cells. This should assist in biochemical characterization of this elusive agent. In other studies, LPVD scientists discovered that recombinant vaccinia virus expressing retroviral envelope proteins induces protective immunity in mice challenged with Friend murine leukemia virus.

NIAID-IRP remains a world leader in basic immunologic research. Research by the Laboratory of Immunogenetics continues to define multigene families that are involved in the control of immune function with emphasis on the structure and function of these genes and their products as well as mechanisms for regulation of these genes. Studies by the Laboratory of Immunology and the Laboratory of Immunoregulation, using monoclonal antibodies, molecular genetics and long-term lines of cloned normal and transformed lymphocytes, have furthered basic understanding of the activation, function, differentiation and regulation of T and B cell receptor genes, as well as other elements of the immune response. Clinical and basic research in the Laboratory of Clinical Investigation have significantly contributed to understanding the pathogenesis of immunologic diseases and provided rational and effective interventions in certain disorders, including allergic diseases. Studies of patients with

phagocyte defects have defined several categories of patients with chronic granulomatous disease of childhood (CGD). Particular attention is now being placed on the feasibility of gene therapy in certain CGD patients.

Equal Employment Opportunity and Affirmative Action Programs have been given considerable attention by the Director, IRP, during the past year. For example, minor deficiencies in promotion practices within NIAID have been identified and steps to correct these deficiencies have been initiated. Conscious efforts to recruit minorities have resulted in the addition of a number of minority staff members and fellows over the past year. The NIAID-IRP Introduction to Biomedical Research Program was held in April, 1986. Due to problems with full-time equivalent cuts and reductions in the operating budget, it was not possible for NIAID to support summer work fellowships. However, a generous gift from Ciba-Geigy made it possible to support 12 summer fellowship students and 10 of these students were minority.

Plans for the renovation of Building 4 on the Bethesda campus have been completed. It is hoped that renovations will start soon. We expect to move into Building 4 in the summer of 1988, at which time Building 5 will be vacated for renovation. Plans have also been completed for replacement of the air handling system in Building 550 in Frederick, MD, and work is expected to start in December, 1986 and to be completed by June, 1987.

The NIAID Board of Scientific Counselors reviewed the Laboratory of Biology of Viruses, the Laboratory of Molecular Microbiology, the Laboratory of Viral Diseases and the Laboratory of Clinical Investigation during the past year. The high quality of scientific research in the IRP continues to be recognized in these evaluations and continued support for each of the above programs was enthusiastically recommended. This year, Paul G. Quie, M.D., completed his tour as Chairman of the Board of Scientific Counselors, and his devotion to the Board and service to the Institute was recognized. The new Chairman of the Board is Robert B. Couch, M.D.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00417-03 OSD
PERIOD COVERED <u>October 1, 1985 to September 30, 1986</u>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>Systematics and Vector Relationships of Ticks (Ixodoidea)</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: James E. Keirans Research Entomologist OSD, NIAID		
COOPERATING UNITS (if any) <u>Dr. C. E. Yunker, Heartwater Project, Harare, Zimbabwe; Drs. D. Sonenshine and P. Homsher, Old Dominion Univ., Dr. J. B. Walker, Onderstepoort, R.S.A., Dr. R. Pegram, Tick Diseases Unit, Lusaka, Zambia.</u>		
LAB/BRANCH <u>Office of Scientific Director</u>		
SECTION <u>Entomology Department, Museum Support Center, Smithsonian, Wash., DC</u>		
INSTITUTE AND LOCATION <u>NIAID, NIH, Bethesda, Maryland 20894</u>		
TOTAL MAN-YEARS: <u>2.0</u>	PROFESSIONAL: <u>1.0</u>	OTHER: <u>1.0</u>
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The activities of this research project currently comprise of three main functions: (1) Specific identification of ticks received from individuals, governmental agencies and universities throughout the world; (2) Systematic investigations, including taxonomy and classification of the Ixodoidea, worldwide; (3) Entry, retrieval and use of tick data in the Smithsonian computer system.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00172-08 OSD
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Structure and Synthesis of Peptides		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Walter Lee Maloy Expert OSD, NIAID Others: John E. Coligan Research Chemist LIG, NIAID		
COOPERATING UNITS (if any) Thomas Kindt, LIG, NIAID; Louis Miller, LPD, NIAID; Ronald Schwartz, LI, NIAID; David Margulies, LI, NIAID		
LAB/BRANCH National Institute of Allergy and Infectious Diseases		
SECTION		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 3.0	PROFESSIONAL: 1.0	OTHER: 2.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided) Within the last year we have synthesized 175 peptides for use in 40 separate studies. These studies are run by 29 investigators. The majority of the peptides have been used to prepare anti-peptide antisera in rabbits. Anti-peptide sera reactive with the C-terminus of the H-2K ^b molecule has been used to investigate alternative RNA splicing patterns in various mouse strains and cell types. Anti-peptide sera made against the constant region of the alpha, beta, and delta chains of the human T-cell receptor complex is being used to examine expression and assembly of the complex. Anti-peptide sera against the alpha and beta chains of the human class II molecules DR, DQ, DP and DO has been prepared and is being used to investigate expression and association of these molecules. An association of I-A and I-E molecules has been observed in the mouse and the nature of this association is being investigated with anti-peptide sera reactive with the I-A and I-E alpha and beta chains. In addition to using peptides to make antisera, peptides have also been used to map determinants recognized by antisera made against intact proteins. This approach has been used for monoclonals against the <u>Plasmodium vivax</u> , <u>Plasmodium berghei</u> and <u>Plasmodium yoelli</u> sporozoite surface antigens. Finally, peptides have been used to define T-cell epitopes in cytochrome C and the <u>Plasmodium falciparum</u> sporozoite surface antigen.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00383-03 OSD
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Acquisition of Specimens from Cases of Acquired Immune Deficiency Syndrome		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Lois A. Salzman, Ph.D., OSD/NIAID		
COOPERATING UNITS (if any) Dr. Louis Baker, New York Blood Center; Dr. Jonathan Gold, Memorial Sloan-Kettering Cancer Center		
LAB/BRANCH Office of the Scientific Director, NIAID		
SECTION		
INSTITUTE AND LOCATION National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 0.5	PROFESSIONAL: 0.5	OTHER:
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <div style="height: 300px; border: 1px solid black; margin-top: 10px;"></div>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00421-02 OSD
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Analysis of Specimens Collected from Populations at Risk of AIDS		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) M.J. Waxdal, Senior Investigator, OSD/NIAID Lois Salzman, Contract Officer, OSD/NIAID Albert Saah, Medical Epidemiologist, ESB/MIDP/NIAID Richard Kaslow, Chief, EBS/MIDP/NIAID		
COOPERATING UNITS (if any) Louis Baker, N.Y. Blood Center; Jonathan Gold, Memorial Sloan-Kettering; Roger Detels, UCLA; David Ostrow, Howard Brown Memorial Clinic, Chicago; Frank Polk, Johns Hopkins; Charles Renaldo, U. Pittsburgh; Warren Winkelstein, UC, Berkeley; Jim Leaf, BRI, Rockville, MD		
LAB/BRANCH Office of the Scientific Director, NIAID		
SECTION		
INSTITUTE AND LOCATION National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS 0.6	PROFESSIONAL 0.6	OTHER
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <div style="height: 300px; border: 1px solid black; margin-top: 10px;"></div>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00228-04 OSD

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Flow Cytometric Analysis of Cell Membrane Antigens & Receptors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

M.J. Waxdal, Senior Investigator, OSD/NIAID

Ron Fico, CSC/DCRT

COOPERATING UNITS (if any)

Claudine Kieda and Michel Monsigny, Centre National de Recherche Scientifique,
Orleans, France; Nathan Sharon, Weitzman Institute, Rehovoth, Israel

LAB/BRANCH

Office of the Scientific Director, NIAID

SECTION

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

2.1

PROFESSIONAL:

0.3

OTHER:

1.8

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00182-08 OSD
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Biochemical & Genetic Mechanisms of Obligate Intracellular Parasitism		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) J. C. Williams, Ph.D., Sr. Scientist, NIAID/OSD, Chief, Rickettsial Diseases Laboratory, USAMRIID E. H. Stephenson, DVM, Ph.D., Col, VC, Chief, Airborne Diseases Division, USAMRIID M. H. Vodkin, Ph.D., USAMRIID C. E. Snyder, Jr., Ph.D., CPT, MSC, USAMRIID		
COOPERATING UNITS (if any) K.-I. Amano, Hirosaki University School of Medicine, Hirosaki, Aomori, Japan V. N. Reinhold, Department of Biological Chemistry, Harvard Medical School, Boston, MA		
LAB/BRANCH Office of the Director of Intramural Research Programs		
SECTION		
INSTITUTE AND LOCATION National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20204		
TOTAL MAN-YEARS 2.0	PROFESSIONAL 1.5	OTHER .5
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> Mechanisms of phase variation in <u>Coxiella burnetii</u>, the etiological agent of Q-fever, were studied using variously virulent strains with different lipopolysaccharide structures to obtain possible molecular correlates of attenuation. A. Genetic heterogeneity. Chromosomal DNA from the Nine Mile phase I strain of <u>C. burnetii</u> (CB9MIc7) cloned into the cosmid vector pHc79 was used as a probe to show a Hae III fragment present in the parent strain but absent from a spontaneously derived Nine Mile phase II strain (CB9MIIC4). An 18 kb deletion in the chromosomal DNA of CB9MIIC4 was identified. Another intrastain spontaneous derivative, CB9MI514, also lacked the sentinel Hae III fragment. This strain carried a deletion which was approximately 29kb, and both deletions appeared to share a common terminus within the limits of resolution. In all other strains investigated, both phase I and phase II, the DNA represented by the insert seemed grossly intact. B. Lipopolysaccharide. LPSs extracted from nine strains of <u>C. burnetii</u> were analyzed for chemical compositions, molecular heterogeneity by sodium dodecyl sulfate polyacrylamide gel electrophoresis, and lethal toxicities in galatamine sensitized mice. The structure of a unique disaccharide prepared from the CB9MIc7 strain was determined using negative ion extraction fast atom bombardment mass spectrometry of the N-acetylated disaccharide, direct chemical ionization mass spectrometry of the N-acetylated then permethylated disaccharide, and DCI and FAB mass spectrometry of reduced permethylated and peracetylated derivatives. The chemical structure was described as galactosaminuronyl-α-(1-6)-glucosamine [GalNU-α-(1-6)-GlcN], [C₁₂H₂₂N₂O₁₀] and Mr of 354. Using GalNU-α-(1-6)-GlcN and two recently described sugars, virenose and dihydrohydroxystreptose, as biochemical markers of truncated LPSs, we were able to relate Mr of selected molecular species of LPSs to truncation of LPS of <u>C. burnetii</u> intra- and inter-specific strains. Smooth-type LPS contained all three compounds, semi rough LPS did not contain virenose, and rough LPS was deficient in all three components. All of the LPSs were toxic in galactamine sensitized mice albeit they were 100- to 1000-fold less toxic than <u>Escherichia coli</u> and <u>Salmonella typhimurium</u> endotoxins. Significance: Identification of chromosomal and plasmid DNA participating in virulence expression and LPS biosynthesis will facilitate our understanding of phase variation and endotoxin activities of <u>C. burnetii</u>. </p>		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00183-08 OSD

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunologic Properties of Coxiella burnetii (Q-fever) Vaccine

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

J. C. Williams, Ph.D., Sr. Scientist, OSD, Chief, Rickettsial Diseases
Laboratory, USAMRIID

E. H. Stephenson, DVM, Ph.D., Col, VC, Chief, Airborne Diseases Division, USAMRIID

C. E. Snyder, Jr., Ph.D., CPT, MSC, USAMRIID

G. H. Scott, Ph.D., USAMRIID

D. M. Waag, Ms. Ph.D., Candidate, OSD, NIAID

C. Bolt, MS, SGT, USAMRIID

J. Beveridge, MS, GS9, USAMRIID

COOPERATING UNITS (if any)

LAB/BRANCH

Office of the Director of Intramural Research Programs, NIAID, Bethesda, MD

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:

4.5

PROFESSIONAL:

2.5

OTHER:

2.0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Immunogenic and pathogenic subfractions of Coxiella burnetii, the etiological agent of Q fever, are being studied in animals and in man in order to evaluate humoral and cellular mechanisms of pathogenesis. A. Mice. Sensitive (A/J) mice develop T-cell suppression and produce anti-lipopolysaccharide (LPS) antibodies late (35 days) in a low dose phase I infection; whereas, resistant (C57BL/6J) mice do not develop T-cell suppression and they produce anti-LPS antibodies early (21 days). Some phase I cells possess an immune suppressive complex (ISC) which consists of three separable components that may not be active alone but components 1 & 2 are required for activity, and component 3 anchors 1 & 2 to the cell surface. The biological response modification (BRM) induced by the ISC favors the growth of C. burnetii while not debilitating the hosts' response to other pathogenic agents. The ISC induces T- and B-cell antigen-specific suppressors which bind antigen. A T-cell suppresses the ConA response while a B-cell suppresses responses to antigen, but in concert these cells participate in an antigen-dependent suppressor circuit. B. Subunit vaccine. Major outer membrane proteins were identified and chromosomal DNA encoding at least 5 proteins were cloned and expressed in Escherichia coli. The structure of one of these proteins may be regulated by the acid environment of the phagolysosome thus conferring acidophilic properties to the C. burnetii cell surface. C. Vaccine in man. A new phase I subunit vaccine designated as chloroform-methanol residue (CMR) produced by the Salk Institute will enter preclinical trials in September 1986. Previously, we showed that the CMR vaccine was non-toxic and efficacious in animals. Significance: The objectives of this project are to define the genetic basis of susceptibility to infection in the mouse model, to characterize components of the ISC, to develop a new recombinant subunit vaccine, and to test the safety and efficacy of the CMR vaccine. Accomplishment of these objectives will allow us to produce recombinant vaccine against Q fever and test the Biological Response Modification in model systems.





LABORATORY OF BIOLOGY OF VIRUSES

1986 Annual Report

Table of Contents

Z01 AI
Project Number

SUMMARY	2-1
00290-05	Mutations in Control Regions that Regulate SV40 Transcription	2-8
00292-05	Factors Required for Specific Transcription	2-9
00293-03	Characterization of Proteins Associated with the Parvovirus, KRV, and its DNA	2-10
00294-05	Structure and Function of Adenovirus DNA	2-11
00295-05	Helper Factors Required for Expression of the Adeno-Associated Virus Genome	2-12
00296-05	Characterization and Production of Parvovirus Proteins	2-13
00297-05	Mechanism and Regulation of Adeno-associated Virus DNA Replication	2-14
00414-02	JC Virus - A Human Virus that Replicates Efficiently in Brain Cells	2-15
00446-02	Properties of RNA Polymerase II	2-16

LABORATORY OF BIOLOGY OF VIRUSES

National Institute of Allergy and Infectious Diseases

SUMMARY - October 1, 1985 - September 30, 1986

During the past year, studies in the Laboratory of Biology of Viruses have been concerned with four groups of DNA containing nuclear viruses, SV40, Adeno-associated viruses (AAV), Herpesvirus, and Adenovirus. The studies have focused on two processes that are critical in virus replication, namely, transcription and DNA replication.

Regulatory mechanisms that function to control viral and cellular genes share a number of common properties. What is unique to the virus is that when it goes through a lytic cycle of growth, a single virus particle gives rise to 10,000 progeny virus particles and so strong selective evolutionary forces are operative on viruses. As a consequence, organization of genetic information in viruses will be unusually efficient and their regulatory structures anticipate what may ultimately occur with higher forms of life.

The precise localization of the start sites for transcription of specific genes has permitted us to identify regions that lie upstream from the start sites which regulate the rate of transcription. The localization of these control regions has allowed us to enhance or suppress expression of specific genes by introducing site specific mutations into these control regions. We have carried out these studies to define the regulatory signals in simian virus 40 and in the control region of the adenovirus major late promoter. With the adeno-associated viruses there is remarkable efficiency in the use of an apparent limited amount of genetic information. This has been achieved by the use of novel patterns of splicing to generate a broad array of messenger RNAs and by the use of an alternative triplet to position that start site of transcription.

All of these studies have provided important new insights as to how genetic information is selectively transcribed and temporally expressed.

Laboratory of Biology of Viruses

National Institute of Allergy and Infectious Diseases

SUMMARY - October 1, 1985 - September 30, 1986

HONORS AND AWARDS

Dr. Norman P. Salzman was appointed to the Editorial Board of Molecular and Cellular Biology and continued to serve on the Editorial Board of the Journal of Virology. He served as Professorial Lecturer, Georgetown University School of Medicine and Dentistry, Department of Microbiology, and was appointed to the Fogarty Scholars Advisory Committee. He served on the Organizing Committee for the NIH-Pasteur Institute Centennial Symposium. He served as Ad Hoc member of the National Institute on Aging Study Section. He chaired a session at the Fogarty sponsored meeting entitled, "Trans-activators of Gene Transcription Involved in Transformation," and coauthored a paper at that meeting.

Dr. Salzman edited two volumes for the series, The Viruses, "The Papovaviridae, the Polyomaviruses" (Ed. N.P. Salzman) and "The Papovaviridae, the Papillomaviruses" (Eds. N.P. Salzman and P. Howley), Plenum Press, in press.

Dr. James Rose continued to serve as an Associate Editor of the journal, Virology (January, 1984) and was appointed to the Editorial Board of Intervirology. He presented an invited lecture at the EMBO Workshop on Parvoviruses in Grangeneuve, Switzerland and coauthored two papers presented at that meeting. He was appointed (for a second tenure) as a member of the NIAID Clinical Research Subpanel.

Dr. Venkatachala Natarajan was a guest speaker at the Laboratory of Developmental Pharmacology, National Institute of Child Health and Human Development, and the Laboratory of Cellular and Developmental Biology, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases. He was an invited speaker at the Fogarty sponsored meeting entitled, "Trans-activators of Gene Transcription Involved in Transformation.

ADMINISTRATIVE CHANGES

Dr. Pierre May and Dr. Evelyne May of the Institute de Recherches Scientifiques Sur le Cancer, Villejuif, France, have been associated with the Laboratory of Biology of Viruses this year. Dr. Pierre May is a Fogarty Scholar and Dr. Evelyne May is a Visiting Scientist.

Some of the highlights of this year's research efforts are detailed below.

A. Structure of the SV40 Promoter Region

In the SV40 early promoter region, we have shown the critical role for the GC base clusters that lie upstream of the TATA box between nucleotide positions 40 and 103. We have recently carried out a study to determine if the base sequences between the GC promoter elements (at np 40-103) and the TATA box (at np 14-21) is also a part of the promoter and whether the third T antigen binding site also functions in autoregulation of the SV40 early promoter.

In bacteria, varying the distance between the Pribnow box at -10 and the -35 upstream element alters the level of transcription. We have found similar results apply to the effect of distance between the TATA box and the first GC cluster. Small deletions or insertions in this region (at np 37) alter the levels of transcription. A four base pair deletion enhances the level of transcription four to six-fold in vitro, but causes a four-fold suppression of gene expression in vivo. These opposite effects can be explained by changes in spacing between the TATA box and the GC rich motifs under in vivo and in vitro conditions. The findings indicate that transcription factors may require simultaneous contact at these two promoter elements.

The six GC clusters have been shown in several studies to be required for SV40 early gene expression both in vivo and in vitro and they are also required for late transcription in vitro. We have shown they could function as independent control elements and that start sites for transcription can be specified by three GC clusters but that a single GC cluster is not sufficient to position the start site of transcription. (May, Nandi and Salzman)

B. The Three AAV Virion Proteins Have Been Mapped on the Viral Genome

The AAV are defective parvoviruses whose replication depends upon a helper adenovirus or herpesvirus. AAV virions contain either a plus or minus DNA strand ca. 4.7 kilobases (kb) in length and are constructed with three polypeptides (A, B, and C) plus at least four additional minor subspecies (one of A and three of C). For AAV2, the apparent sizes of the primary capsid components are 90 (A), 72 (B), and 60 (C) kilodaltons (kd). Previous studies from our laboratory have revealed that these proteins (i) contain overlapping amino acid sequences, (ii) that they are encoded in the right half of the virus genome, and (iii) that one or both of the smallest viral RNA species (2.3 or 2.6 kb RNA) account for their synthesis. We have now shown that protein A originates from one of two ATG sites within the intervening sequence, which is intact in the larger (unspliced) 2.6 kb mRNA. Either a read-through of a strong stop signal (UAA) or a frame-shift is required for its production. Proteins B and C are not derived from protein A but originate from independent, in-frame initiations that lie downstream from the splice junction. Of considerable interest is the finding that protein B initiates from an unexpected initiation codon (ACG) that lies 64 codons upstream from the start site (AUG) for C. Production of the three AAV capsid proteins must involve at least two mRNA species. The B and C

proteins arise from the spliced 2.3 kb RNA, whereas protein A should be generated by the 2.6 kb RNA or a hitherto unidentified spliced RNA species. (Becerra, Sebring and Rose)

C. Identification of Genes that Specify Nonstructural AAV Proteins

Regarding the nonstructural AAV proteins, *in vitro* translation of mRNA selected by the entire AAV2 genome gives rise to seven or eight polypeptides in addition to the three structural proteins. Messages selected with fragments of DNA from the left half of the genome do not translate the three structural proteins, but on translation *in vitro*, yield proteins with molecular weights of 70 kd and 50 kd as well as several smaller polypeptides (molecular weights ranging from 15 to 42 kd). On the basis of both hybrid-selection and hybrid-arrest studies, most of these proteins could be assigned to RNA species arising from the first (p5) or second (p19) promoter. The largest protein (70 kd) and possibly most of the smaller polypeptides (15-26 kd) are specified by p5 RNA, whereas the 50 kd protein is generated by p19 messages. It is interesting that the 70 kd protein is about the size of the larger of two nonstructural proteins now known to be produced by several autonomous parvo-viruses. This protein apparently acts as a trans-acting stimulator of capsid protein transcript production. The smaller nonstructural autonomous parvovirus protein is about 25 kd, a size present in our small polypeptide population, and it is also comparable in size to a 25 kd *in vivo* synthesized nonstructural AAV protein we described previously. This protein might play a role in DNA replication (see DNA synthesis project). (Becerra, Sebring and Rose)

D. Nonstructural Proteins Specified by the Viral Genome Function during AAV DNA Replication

The AAV genome is either a plus or a minus linear single-stranded DNA with partially palindromic inverted terminal repeats. In the case of AAV type 2 (AAV2), the lengths of the genome and the inverted terminal repeat are 4,675 and 145 nucleotides. The 125 base-long palindromic sequence within the terminal repeat can form a T-shaped hairpin structure. The overall scheme of AAV DNA synthesis *in vivo* was first described in our laboratory. Briefly, following coinfection of KB cells with AAV and a helper Ad, AAV DNA synthesis is initiated on single-stranded genomic templates and proceeds by a self-priming mechanism with the 3' terminal hairpin serving as the primer. Subsequent elongation yields a unit length hairpin intermediate. A second round of self-primed synthesis displaces the 5'-ended arm of the hairpin and leads to either (i) displacement of a complete plus or minus progeny strand (by virtue of a processing/synthesis step at the closed end of the hairpin) or (ii) concatemeric molecules (if closed end processing/synthesis does not occur) which are eventually processed into unit length duplex templates. The mechanism of self-primed DNA synthesis thus requires origin-specific, single-stranded cleavages in hairpin termini and at similar internal sites in concatemeric intermediates to yield unit-length duplex templates from which progeny strands can be generated by displacement synthesis. To identify the predicted factor(s) responsible for the site-specific processing reaction, we analyzed (i) DNA products synthesized in cell-free extracts with DNA templates obtained from purified AAV2 virions and (ii) the molecular construction of template molecules. When AAV DNA was released in a 4M guanidinium chloride-sucrose gradient, a DNA-protein template component (P2) was obtained which directed synthesis of both unit-length hairpins and completely denaturable nonhairpin duplexes. Upon treatment with proteinase

K, a fraction of the P2 molecules released terminal palindromic fragments, suggesting that these fragments may be linked by a protein at the putative processing site. SDS-PAGE analysis of the P2 molecules revealed two associated proteins, $M_r=25,000$ and $22,000$. These proteins appear to represent new AAV virion polypeptides, and correspond in size to previously observed nonstructural components of AAV and autonomous parvoviruses. We propose that one or both of these proteins may play a role in origin-specific processing during replication of AAV DNA. (Sebring, Ohi, Wong and Rose)

E. Identification of Genes in Helper Viruses that are Required for AAV Replication

Parvoviruses are among the smallest DNA-containing animal viruses. They are assembled in the cell nucleus and are approximately 20 nm icosahedral particles with a single-stranded DNA genome. Their relatively high DNA content (20-25%) produces a characteristically high buoyant density in CsCl ($1.40-1.45 \text{ g/cm}^3$). In general, they are very durable agents (heat and ether resistant), and numerous species have been found to infect a wide variety of animal hosts, including man. Pathogenic feline, bovine, porcine, mink, canine and human parvoviruses are known to exist. In addition, viral persistence and latency (at least in the case of the adeno-associated viruses [AAV]), the AAV genome is readily integrated into and rescued from cellular DNA) are characteristic of parvoviruses and may be eventually biologically important (e.g., related to slow virus disease). There are two major virus groups, (A) nondefective and (B) defective (AAV) parvoviruses. The AAV multiply only when cells are also infected with a helper virus (either an Ad or a herpesvirus).

We have previously demonstrated that several early Ad genes (i.e., E1A, VA RNA I or II, E2A, E4) are required for replication of the AAV. DNA transfection studies have indicated the roles (at least in part) played by the Ad VA RNA(s) and DNA-binding protein (2A gene product). It is clear that efficient translation of the AAV capsid proteins requires the presence of both these Ad factors. Similar analyses to define which cloned segments of herpes simplex virus (HSV) and cytomegalovirus DNA are required for AAV replication are now in progress. It will be important to compare the required helper virus genes with respect to genomic organization, specific functions(s) and cross-homologies. (Lalji and Rose)

F. Factors that Determine Permissivity of Adenoviruses and Adeno-associated Viruses in Monkey Cells

It has been recognized for many years that SV40 coinfection overcomes the host range (hr) restriction (transcriptional/translation) of monkey cells for human Ad (and AAV) replication. We have now found, by use of DBP (Ad DNA-binding protein gene) deletion mutants, that this enhancement does not occur as a direct substitution of the SV40 T-antigen for the Ad DBP as previously suggested. Our present findings strongly support the interpretation that SV40 T-antigen either directly modifies the DBP chemically or in some way may alter the interaction of a host cell factor(s) with DBP.

Newly developed DBP mutants now provide a means to analyze functions of each domain of the Ad DBP. The DBP has two distinct domains: an amino

(N)-terminal smaller segment in which an hr mutation (permissive for replication) occurs and a carboxyl (C)-terminal larger portion which binds to SS-DNA. These two regions appear to have separable functions with the C-domain participating in DNA synthesis and the N-domain affecting transcription and translation. Studies, to define the role of the N-domain in translational regulation *in vivo* using AAV protein synthesis as a marker for DPB function in coinfections with certain Ad mutants are planned. The use of AAV enables us to explore the role of the DPB in translation independent of its affect on DNA synthesis. This strategy is not possible in Ad infections alone since these DBP mutants contain C-domain deletions which would also restrict Ad DNA synthesis and hence affect late transcription and translation. (Rose)

G. Regulation of Adenovirus Gene Expression

The IVa₂ promoter is not expressed *in vitro* with the Manley transcription system. However, we have been able to transcribe the IVa₂ promoter *in vitro* by including ammonium sulfate in the reaction mixture. To define the control sequences of this promoter, a series of promoter deletion mutants was constructed and then transcribed *in vitro*. By this approach, two sequences have been identified, both rich in cytidine, one proximal to the RNA initiation site at nucleotide positions -38 to -49, and a distal domain between nucleotide position -152 and -242 as essential for IVa₂ transcription where the IVa₂ RNA initiation site is nucleotide position +1. Transcription efficiency is decreased by 70-90% after the deletion of the proximal domain when either linear or supercoiled DNAs were used as template. However, distal sequences functioned as a transcriptional control domain only with a covalently closed DNA template. The deletion of both the proximal and distal regions from covalently closed DNA templates reduces the level of IVa₂ transcription by a factor of 100-500.

The distal control region of the IVa₂ promoter has been further resolved into at least two domains. The first distal domain, present between np -152 and -179, is necessary for efficient transcription of the IVa₂ promoter, and it overlaps with sequences that have been shown to be necessary for efficient transcription of MLP. This region may serve as the entry site for the transcription machinery. The second distal domain consists of sequences present between np -211 and -242. These sequences are contained at the 5' end in the MLP transcript, and they inhibit transcription from the IVa₂ promoter. However, these sequences are not necessary for transcription of the MLP with a covalently closed template but are needed for transcription with a linear template. The TATA box that is located at np -180 to -186 between these two domains has a critical role for efficient transcription of the MLP. A point mutation that reduces transcription from MLP by more than 80% stimulates transcription from IVa₂ by ten-fold. These findings are consistent with the proposal that MLP and IVa₂ promoters share an entry site for transcription machinery and compete for its use.

The IVa₂ promoter control sequences have also been analyzed *in vivo* by using this promoter to a bacterial gene which codes for chloramphenicol acetyl transferase (CATase) and transfecting cells in culture. Synthesis of IVa₂ specific RNA was dependent on plasmids containing an enhancer that was present in cis. When HeLa cells were transfected with various deletion mutants with an enhancer in cis, it was seen that sequences -38 to -64 base

pairs upstream of the RNA initiation site are necessary for efficient transcription, suggesting that the sequence present -38 to -49, identified as a proximal control element in vitro is likely the same domain that is important for transcription in the transient assay in vivo. The distal control sequences between np -152 and -242 that is required in vitro was not necessary for in vivo transcription. The requirement for the distal control region may be masked by the presence of the enhancer that is needed in cis for expression of the IVa₂ promoter. However, during the course of the adenovirus infectious cycle, it is likely that the distal control sequence functions in controlling transcription from the IVa₂ promoter.

By using the band competition assay of Strauss and Varshavsky, we have identified a factor which binds to the IVa₂ promoter. Competition studies with various deletion mutants demonstrate that the region present 152 to 160 base pairs upstream of the IVa₂ transcription start site is necessary for binding of this factor. DNase-I footprinting assays demonstrate directly that this factor interacts with the distal domain of the IVa₂ promoter. This factor is necessary for efficient transcription from the IVa₂ promoter as evidenced by the ability of a DNA fragment containing the binding site for this factor to inhibit transcription. Its role in bidirectional activation of transcription from major late and IVa₂ promoters may be as an entry site for the transcription machinery.

The adenovirus Ela gene product has been shown to activate other adenovirus genes. In the absence of enhancer sequences, cotransfection to provide the adenovirus Ela gene in trans also stimulated IVa₂ RNA synthesis. The Ela gene in trans and an enhancer in cis have an additive effect on RNA synthesis from both IVa₂ and major late promoters. (Natarajan, Vasudevachari, Singh and Salzman)

H. Monoclonal Antibodies that are able to Block Transcription by RNA Polymerase II have been Prepared

We had previously examined in vitro transcription with purified RNA polymerase II using a DNA template that contains the adenovirus major late promoter. Transcription started at the specific initiation site that was within the TATA box that lies 30 base pairs upstream of the authentic in vivo start site. This finding does not reflect an artifactual pattern of binding since sequences identical to this TATA box are present at two other sites in the plasmid but initiation did not occur at either of these sites. It suggests that the recognition site of the enzyme is the TATA box and its surrounding sequences.

Our present studies have involved large scale purification of RNA polymerase II. This work is currently in progress with the goal of providing partial amino acid sequences of certain of the subunits. We have prepared a series of monoclonal antibodies after immunization of Balb c mice with purified RNA polymerase II. Two well characterized monoclonal antibodies have been obtained. They are both able to inhibit in vitro transcription. It has not yet been established which RNA polymerase II subunit reacts with the monoclonal antibody. (Selzer, May, Xu and Salzman)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00290-05 LBV
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Mutations in Control Regions that Regulate SV40 Transcription		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Norman P. Salzman, Ph.D. Others: Evelyne May, Ph.D. Visiting Scientist LBV, NIAID		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Biology of Viruses		
SECTION Biochemical Virology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205		
TOTAL MAN-YEARS. 2.0	PROFESSIONAL: 1.4	OTHER: .6
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p style="margin: 10px 0;"> A 400 base pair region in SV40 contains the promoter elements of early and late transcription and the origin of DNA replication. We have identified a surrogate TATA box that lies 30 base pairs upstream of the 5' start site of late transcription that functions <u>in vitro</u> and <u>in vivo</u> to regulate the level of late transcription. The level of early transcription is effected by GC rich sequences and their distance from the TATA box that lies upstream of the early 5' start site. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00292-05 LBV
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Factors Required for Specific Transcription		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Norman P. Salzman, Ph.D. Chief LBV, NIAID V. Natarajan, Ph.D. Visiting Associate LBV, NIAID Others: R. Singh, Ph.D. Visiting Fellow LBV, NIAID M. B. Vasudevachari Ph.D. Visiting Fellow LBV, NIAID		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Biology of Viruses		
SECTION Biochemical Virology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205		
TOTAL MAN-YEARS: 6	PROFESSIONAL: 4	OTHER: 2
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> DNA sequences that function as promoter elements for the adenovirus IVa₂ gene have been characterized. Promoter elements are shared by the IVa₂ gene and the adenovirus major late promoter whose 5' start site lies 210 base pairs from it and is transcribed from the opposite strand. The two genes compete for transcription factors and when expression of the major late promoter is blocked by point mutations, there is a ten-fold enhancement of IVa₂ gene expression. The role of trans acting factors in the regulation of gene expression has been examined and one cellular protein that binds to this promoter region has been identified. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 A1 00294-05 LBV
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Structure and Function of Adenovirus DNA		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: James A. Rose, M.D. Section Head LBV, NIAID		
COOPERATING UNITS (if any) (1) Richard McPherson, M.D., Department of Pathology, Georgetown University Hospital, Washington, D. C., (2) Daniel Klessig, University of Utah School of Medicine, Salt Lake City, Utah		
LAB/BRANCH Laboratory of Biology of Viruses		
SECTION Molecular Structure Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205		
TOTAL MAN-YEARS: .7	PROFESSIONAL: .3	OTHER: .4
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) <p> Among objectives of these studies has been the application of physical, biochemical and biological techniques to characterize the structure and functions of certain segments (i.e., the inverted terminal repeat) and genes of adenovirus (Ad) DNA. In earlier studies, we first identified and characterized the <u>VA RNA gene/transcript and inverted terminal repeats</u> in Ad DNA. We have continued to investigate the specific regulatory functions of several early Ad genes, e.g., the <u>VA and DNA-binding protein genes</u>. Our results indicate that these latter genes are involved in the <u>regulation of translation</u> of certain viral mRNAs. Among methods used are <u>gradient sedimentation</u>, <u>DNA cleavage with restriction endonucleases</u>, <u>gel electrophoresis</u>, <u>base sequence analysis</u> and <u>DNA transfection</u>. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 A1 00295-05 LBV
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Helper Factors Required for Expression of the Adeno-Associated Virus Genome		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: James A. Rose, M.D. Section Head LBV, NIAID Others: Lalji Mishra, Ph.D. Visiting Associate LBV, NIAID		
COOPERATING UNITS (if any) Richard McPherson, M.D., Dept. of Pathology, Georgetown Univ. Hosp., Wash., D. C., Leonard Rosenthal, Ph.D., Dept. of Microbiology, Georgetown Univ., Wash., D.C., John Hay, Ph.D., Dept. of Microbiology, Uniformed Services University of the		
LAB/BRANCH Health Sciences, Bethesda, MD Laboratory of Biology of Viruses		
SECTION Molecular Structure Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205		
TOTAL MAN-YEARS 1.2	PROFESSIONAL: .3	OTHER: .9
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The main objectives of this project are (i) to define where and how each required <u>helper virus factor regulates expression of defective human parvovirus (AAV) genomes</u> and (ii) to relate these findings to their respective roles in the replication of the <u>helper viruses (adenoviruses, herpesviruses)</u> themselves as well as to potentials for <u>selective interference with viral infection</u>. We previously mapped the adenovirus genes required for AAV replication and continue to investigate their specific helper functions. Similar studies are in progress with herpes simplex viruses. Among methods used are specific immunofluorescence, cleavage of DNA with <u>restriction endonucleases</u>, DNA cloning, <u>gel electrophoresis</u>, <u>blot-hybridization</u> analyses and <u>DNA transfection</u> of cells. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 A1 00296-05 LBV
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Characterization and Production of Parvovirus Proteins		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: James A. Rose, M.D. Section Head LBV, NIAID Others: Edwin Sebring, Ph.D. Research Chemist LBV, NIAID Patricia Becerra, Ph.D. Visiting Associate LBV, NIAID		
COOPERATING UNITS (if any) Carl W. Anderson, Ph.D., Brookhaven National Laboratory, Upton, New York 11973		
LAB/BRANCH Laboratory of Biology of Viruses		
SECTION Molecular Structure Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205		
TOTAL MAN-YEARS: 2.3	PROFESSIONAL: 1.7	OTHER: .6
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) <p> The main objectives of these studies are (i) to identify and characterize all <u>proteins</u> that are specified by the defective human parvoviruses (AAV) and to determine similarities and differences with autonomous parvovirus proteins, (ii) to define the mechanism(s) by which the AAV proteins arise and (iii), to define specific functions of the AAV proteins. We have identified several AAV non-structural proteins which were previously undetected. At least one of these proteins is necessary for viral DNA replication. <u>Post-translational processing</u> does not account for production of any AAV structural proteins, although they share large proportions of <u>sequences-in-common</u>. It is now clear, however, that these proteins originate from <u>independent in-frame initiations</u>. The mechanism that <u>regulates translation</u> of AAV proteins is of fundamental interest, and we have now shown that one AAV structural protein is initiated by a codon not known previously to act as an initiation codon in eukaryotes. Furthermore, our current findings support a "<u>scanning mechanism</u>" in the translational expression of polycistronic eukaryotic mRNAs. Among methods used are affinity chromatography, gel electrophoresis, <u>in vitro translation</u> of viral RNA, <u>electrophoretic and HPLC analyses</u> of V8 protease and tryptic peptides and <u>aminoterminal sequencing</u> of purified polypeptides. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 A1 00297-05 LBV
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Mechanism and Regulation of Adeno-associated Virus DNA Replication		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: James A. Rose, M.D. Others: Edwin Sebring, Ph.D. Seigo Ohi, Ph.D. Kamehameha Wong, M.D.	Section Head Research Chemist Visiting Associate Medical Staff Fellow	LBV, NIAID LBV, NIAID LBV, NIAID LBV, NIAID
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Biology of Viruses		
SECTION Molecular Structure Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205		
TOTAL MAN-YEARS: 4.5	PROFESSIONAL: 3.7	OTHER: .8
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The primary objective of this project is to define molecular and biochemical mechanisms involved in <u>eukaryotic DNA synthesis</u>. To approach this problem, we are investigating adeno-associated virus (AAV) DNA replication in both <u>in vivo</u> and <u>in vitro</u> systems. Current results show that AAV DNA synthesis can be initiated <u>in vitro</u> and that replicating forms that correspond to those identified <u>in vivo</u> can be synthesized with either endogenous or exogenously added templates. Recently, we have identified two <u>DNA-associated proteins</u> that may play an important role (one or both proteins) in the <u>required processing</u> of self-primed unit length hairpin and concatemeric intermediates of DNA replication. The observed mode of AAV DNA replication may serve as a model for <u>telomere replication</u> in eukaryotes. Among methods used are <u>affinity chromatography</u>, <u>gel electrophoresis</u>, <u>DNA sequence analysis</u> and <u>restriction cleavage</u> of DNA molecules. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00414-02

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

JC Virus - A Human Virus that Replicates Efficiently in Brain Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Norman P. Salzman, Ph.D.

Others: Shannon Kenney, M.D.

Medical Staff Fellow

LBV, NIAID

V. Natarajan, Ph.D.

Visiting Associate

LBV, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Biology of Viruses

SECTION

Biochemical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00446-02 LBV
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Properties of RNA Polymerase II		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Norman P. Salzman, Ph.D. Chief LBV, NIAID		
Others: Dr. Gerald Selzer Senior Staff Fellow LBV, NIAID Dr. Evelyne May Visiting Scientist LBV, NIAID Dr. Feng-Sheng Xu Visiting Fellow LBV, NIAID		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Biology of Viruses		
SECTION Biochemical Virology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205		
TOTAL MAN-YEARS 3.8	PROFESSIONAL 1.8	OTHER 2.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p style="margin-left: 40px;">A series of monoclonal antibodies directed against RNA polymerase II have been prepared. Three of these lines are able to inhibit transcription using an <u>in vitro</u> assay. The monoclonals, all of which are IgM, have not yet been characterized regarding which subunit they recognize.</p>		



LABORATORY OF CLINICAL INVESTIGATION
1986 ANNUAL REPORT
TABLE OF CONTENTS

<u>Z01-A1</u> <u>Project Number</u>		<u>Page</u>
Summary		3-1
00043-21	Immunology and Chemotherapy of Systemic Mycoses-- Bennett	3-5
00045-18	Studies on Interaction of Antibody and Complement on Production of Immune Damage--Frank	3-6
00047-17	Clinical Studies of Patients with Known or Suspected Parasitic Diseases--Ottesen	3-7
00048-16	The Pathophysiology of Autoimmune Hemolytic Anemia--Frank	3-8
00057-13	Basic Studies on Pathogenic Fungi--Kwon-Chung	3-9
00058-12	The Pathogenesis and Chemotherapy of Herpesvirus Infections in Man--Straus	3-10
00154-11	Events in Immediate Hypersensitivity--Kaliner	3-11
00155-11	Phagocytic Cell Function--Gallin	3-12
00189-07	Clinical and Biochemical Studies of Human Enteral Adenovirus Infections--Straus	3-13
00192-08	Studies on Immediate Hypersensitivity--Metcalfe	3-14
00249-05	The Pathogenesis, Diagnosis, and Treatment of Systemic Mast Cell Disorders--Metcalfe	3-15
00250-05	Clinical and Basic Studies on Inflammatory Diseases of the Gastrointestinal Tract--Metcalfe	3-16
00269-05	Neutrophil Subpopulations--Seligmann	3-17
00270-05	Tubulin Tyrosinolation in Normal and Abnormal Human Neutrophils--Nath	3-18
00271-05	Purification and Characterization of Serum Complement Proteins and Fragments--Hammer	3-19
00272-05	Host Defense Against Pneumococcal Bacteremia-- Brown	3-20

Annual Report (Continued)
Laboratory of Clinical Investigation

<u>Z01-A1</u> <u>Project Number</u>		<u>Page</u>
00273-04	Role of Fibronectin in Opsonization and Phagocytosis--Brown Terminated	3-21
00275-05	The Complement Receptor and C3 Mediated Opsonization--Gaither	3-22
00276-05	Studies of the Membrane Attack Complex of Human Complement--Joiner	3-23
00277-05	Mechanism of Serum Resistance in Bacteria--Joiner	3-24
00278-05	Studies of the Components of the Complement Cascade--Hammer	3-25
00279-05	Studies on Mucous Glycoproteins--Kaliner	3-26
00354-04	Immunoregulatory Defects in Inflammatory Bowel Disease--James	3-27
00355-04	Immunoregulatory Defects in Primary Biliary Cirrhosis--James	3-28
00356-04	Studies of the Regulation of IgA Immunoglobulin Synthesis--Strober	3-29
00357-04	Studies of the Autologous Mixed Lymphocyte Reaction--Strober	3-30
00396-03	Complement Receptors: Regulation of Expression and Cell Biology--O'Shea	3-31
00397-03	Interactions of C3b with Immunoglobulin G-Regulation of C3b Function by Antibody--Fries	3-32
00398-03	Studies on the Interaction of Complement and Parasites--Joiner	3-33
00428-02	IgE Immunotoxins--Kaliner	3-34
00429-02	Studies on Nasal Responses--Kaliner	3-35
00430-02	Molecular Biology of Varicella-Zoster Virus Infections--Straus	3-36
00432-02	Regulation of Mucosal Immune Responses in Non-human Primates--James	3-37

Annual Report (Continued)
Laboratory of Clinical Investigation

Z01-AI		
<u>Project Number</u>		<u>Page</u>
00447-02	Clq: Its Biosynthesis and Biological Functions--Tenner	3-38
00469-01	Complement: Studies in Viral Infection--Fries	3-39
00470-01	A Study of Sporadic Neurasthenia Associated with Epstein-Barr Virus	3-40
00481-01	Receptors and Transduction Mechanisms in Human Phagocytes--Malech	3-41
00482-01	Endothelial Cells in Inflammation--Rotrosen	3-42



SUMMARY OF PROGRAM

Laboratory of Clinical Investigation
October 1, 1985 to September 30, 1986

Michael M. Frank, M.D., Chief of Laboratory
and Clinical Director, NIAID

The structure of the laboratory includes groups working in clinical immunology, allergy and infectious disease. There is considerable interaction between the groups and a large number of collaborative projects are ongoing. Moreover, we have joint laboratory discussion sessions and individuals from different groups with different points of view have helped each other to a major extent.

The allergy group currently has two sections. Dr. Michael Kaliner, head of the allergy program, conducts widely ranging research that extends from the development of ricin-IgE linked immunotoxins, to studies of autonomic nervous system in allergic responses, to studies of blood flow to the mucus membranes of the nose.

Of particular interest are the detailed studies that analyze control of secretion of mucous glycoprotein. The group has shown that phagocytosing macrophages secrete a factor that stimulates mucus secretion. In addition they have shown that the complement protein derived factors C3a and C5a stimulate mucus secretion by a mechanism that does not involve mast cell stimulation. Glucocorticoids inhibit mucus secretion and the group has demonstrated these agents act via stimulation of lipocortin, which in turn inhibits phospholipase A₂ action. Studies of late phase allergic reactions have led to demonstration of a factor derived from human neutrophils that causes certain mast cells and basophils to degranulate. An imaginative set of experiments have utilized the technique of laser-Doppler velocimetry to explore blood flow to the nasal mucosa to help determine the factors that increase or decrease nasal secretion. Using this technique it has been possible to differentiate the factors that control nasal blood flow and secretion of ultrafiltrate from those that control mucus secretion.

Dr. Dean Metcalfe heads the Mast Cell Physiology Section. His interests include studies of the mast cell and its function, studies of peptidoglycan structure and function and studies of food allergy. In particular, the group has described a number of features of mast cell heterogeneity and has identified mast cells and their products in arthritic joints. They have had success in identification and separation of clinical mast cell syndromes and are in the process of developing new methods of specific treatment. His group has received wide recognition for the development of carefully controlled clinical studies of food allergy.

The infectious disease group is involved in a wide range of studies of infectious organisms and phagocytic cells. Dr. John Gallin has developed his interest in phagocytic cell function including the mechanisms underlying the chemotaxis of neutrophils, the physiology of neutrophil function and the mechanism of granule exocytosis. The group has had great success in defining subgroups of patients with genetically different variants of chronic granulomatous disease and has recently described their experience with CR3 receptor deficiency. The latter complement receptor was shown to be located within the specific granule pool of the neutrophil and to be translocated to

the neutrophil surface. Electrophysiologic changes of the cell membrane or stimulation with chemotactic factors have been explored in detail.

Dr. Stephen Straus directs the viral disease program. An additional senior member of the program is Dr. Jeffrey Ostrove, a Ph.D. molecular biologist. The group has succeeded in mapping an entire varicella-zoster genomic library. Sequences have been identified that are strain specific and have allowed for studies of epidemiology, viral transmission and viral reactivation. They have recently completed the first viral transcript map and are now mapping specific viral genes and their products. The group has reported extensive studies of herpes virus infection in man with particular emphasis on the role of acyclovir in genital herpes virus infection. More recently they have begun extensive studies of the Epstein-Barr neusthenia syndrome, defining it as an entity and beginning placebo controlled drug trials.

Dr. John Bennett is in charge of the clinical mycology group. Dr. June Kwon-Chung, one of the country's leading fungal geneticists, is a member of that group. Dr. Bennett is generally recognized as a leading infectious disease clinician. His research interests include studies of the pathogenesis of cryptococcal infection and study of many types of fungal infection for circulating antigen in body fluids to aid in early diagnosis. His group has recently shown that *Aspergillus fumigatus* secretes a factor that blocks the host opsonin C3b. They have studied the interaction of *Aspergillus* conidia with monocytes and macrophages and shown that an important factor in attachment of the conidia to the cells is a lectin like binding inhibitable by chitin constituents, N-acetyl glucosamine and chitotriose as well as by galactomannan. Dr. Kwon-Chung's research has emphasized genetics and factors in pathogenesis of cryptococcal infection. The group continues to extend its observations on new varieties of *Cryptococcus* with new information on the epidemiology of *C. neoformans* var. *neoformans* and *C. neoformans* var. *gattii*. Both the polysaccharide capsule and phenoloxidase have been established as virulence factors. Complementation tests have been developed to explore genetic marker variants in these two groups of organisms. Similar genetic studies have yielded new information on factors important in pathogenicity of *Candida albicans*. Clinically, the mycology group has been interested in the development of new anti-fungal agents like the experimental drug SF 86-327.

A program in host defense against bacterial infection is underway in the Clinical Immunology Section as well under Dr. Keith Joiner and a new member of the senior staff, Dr. Louis Fries. Working with Drs. Frank and Hammer, Dr. Joiner has completed an important series of studies on the mechanism by which complement and antibody act to damage bacteria and the mechanisms that bacteria have evolved to subvert this attack. It has been found that various classes of organisms have evolved different mechanisms for subverting antibody and complement attack. Both serum sensitive and serum resistant enterobacteriaceae allow complement to be deposited on the organism's surface. When the hydrophobic membrane attack complex (MAC) forms upon completion of the complement protein sequence, it successfully inserts in the bacterial outer surface in serum sensitive organisms but is shed from the surface of resistant bacteria. *Neisseria gonorrhoea* have evolved another mechanism. The MAC is sequestered by interacting with specific proteins that prevent successful outer membrane insertion. In addition, these studies indicate that antibody does not serve as a passive activator of the complement cascade. Antibody of the "right specificity" directs stereo-specifically the attack sequence to cause membrane damage. More recently Dr. Joiner has turned his attention to the mechanism by

which antibody and complement attack parasites, defining the proteins to which the components attach and the mechanisms developed to subvert this attack. Dr. Fries has been studying host defense against Herpes simplex virus and has shown that the viral genome codes for a protein, Herpes glycoprotein C, that specifically interrupts the complement cascade, protecting the infected cell.

The clinical immunology area is represented by the sections of Drs. Warren Strober and Michael Frank. Dr. Strober is interested in studies of mucosal immunity with a particular interest in IgA synthesis and the mechanism underlying the differentiation of B lymphocytes into IgA secreting cells. The group has recently demonstrated in the human appendix "switch cells", T cells that are necessary for isotype differentiation of B cells from IgM to IgA secretion. The molecular biology of this process is under study using T cell hybridomas and B cell lymphomas. It has been shown that T cells regulate B cell heavy chain genes at two levels, transcription and post transcriptional translation. All reactive T cells with the Leu 3 positive phenotype have been shown to provide either help or suppression depending on their state of differentiation and how they are stimulated. Dr. Strober is widely recognized for his studies of gluten sensitive enteropathy and is currently engaged in a detailed study of the granulomatous bowel diseases, Crohn's disease and ulcerative colitis. Early Crohn's disease is frequently associated with the presence of a circulating suppressor T cell that appears to arise as a result of an autoreactive interaction with activated B cells or macrophages. Interestingly T cell subsets in Crohn's disease were found to be normal on direct analysis. Dr. Strober is joined in these studies by Dr. Stephen James who also conducts studies of the role of infectious agents like Chlamydia in causing bowel ulceration. Lymphogranuloma venereum infection is being examined in Cynomolgus monkeys. Antigen responsive T cells can be found in blood and peripheral tissues during infection but not in the GI mucosa. Presumably in the bowel all these cells have differentiated into effector cells and can no longer proliferate in response to antigen. Cyclosporin treatment of the monkeys was shown to decrease the immune response of circulating cells but to increase the immune response of cells in the mucosa, which may have an important bearing on the usefulness of this agent in treatment of Crohn's disease.

The Clinical Immunology Section under Dr. Frank is interested in the function of antibody and complement in the host defense process and in causing tissue damage in autoimmune disease. Purification of complement components is essential to these studies and is the responsibility of Dr. Carl Hammer who has developed many of the standard techniques of component purification now widely used. Recent new findings include the development of rapid methods for the purification of human anaphylatoxins, suitable for use in man developed with Drs. T. Lawley and K. Yancey. Studies of these agents in man are ongoing with the interesting observation that C5a in human skin is inefficient in triggering mast cell mediator release, but appears to require neutrophil presence for optimal effect. The presence of a physiologic form of macromolecular, cleaved, C5 with anaphylatoxic activity has also been demonstrated. C6 and C7 are required to release C5a from this macromolecular form. Interest of the group ranges from detailed studies of serum sickness to studies of the basis of paroxysmal nocturnal hemoglobinuria. For the first time serum sickness in man has been studied prospectively with demonstration of new clinical signs of disease, careful follow-up of serologic findings and demonstration of the role of the immune response to heterologous serum protein in generation of arthritis, renal disease and GI bleeding. The fundamental basis of PNH has

been re-explored with the demonstration that progenator cells that divide to form cells with the PNH phenotype are phenotypically normal. These surprising experimental results have led to a rethinking of the pathogenesis of PNH, thought to be a clonal abnormality. Detailed studies of complement receptors, their physiologic function and their role in phagocytosis are underway. As mentioned, Drs. Keith Joiner and Louis Fries participate in many of these studies. A senior technician, Ms. Thelma Gaither, has led a series of studies of phagocytosis of IgG coated particles by normal neutrophils, neutrophils from patients with chronic granulomatous disease and neutrophils from a patient with myeloperoxidase deficiency. In brief, she has shown that normal neutrophils rapidly down regulate their Fc receptor function, probably via products of the oxidative burst and this appears to be a physiologic control mechanism.

In completing this report of LCI activities it is appropriate to mention that of 8 tenured physicians on our staff, six have now been elected to the American Society for Clinical Investigation. Four of our physicians have been elected to the Association of American Physicians. Two are members of the American Board of Allergy and Immunology. Three have served on the editorial board of the Journal of Allergy and Clinical Immunology. Two are section editors of the Journal of Immunology and many serve on the editorial boards of that and other journals.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00043-21 LCI
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Immunology and Chemotherapy of Systemic Mycoses		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: Others:	J.E. Bennett, Head, Clinical Mycology Section R.G. Washburn Medical Staff Fellow V.L. Kan Medical Staff Fellow J.M. Delga Visiting Fellow J.I. Gallin Director C.H. Hammer Senior Investigator K.J. Kwon-Chung Senior Investigator	LCI, NIAID LCI, NIAID LCI, NIAID LCI, NIAID IRP, NIAID LCI, NIAID LCI, NIAID
COOPERATING UNITS (if any) Hospital Epidemiology Service, CC (D.K. Henderson), Microbiology Service, Clinical Pathology Department, CC (M.J. Mitchell), Data Management Branch, DCRT (D.A. George)		
LAB/BRANCH Laboratory of Clinical Investigation		
SECTION Clinical Mycology Section		
INSTITUTE AND LOCATION National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD		
TOTAL MAN-YEARS: 5.0	PROFESSIONAL: 3.0	OTHER: 2.0
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Human peripheral blood monocytes were found to kill <u>Aspergillus fumigatus</u> conidia by two hydrogen peroxide-mediated mechanisms, one being the myeloperoxidase-halide system and the other being the ferrous ion-chloride system.</p> <p>Murine pulmonary alveolar macrophages bound unopsonized <u>Aspergillus fumigatus</u> conidia <u>in vitro</u>. Binding was significantly inhibited by certain sugars and by mild trypsinization of the macrophage. The most effective sugars causing inhibition were <u>Saccharomyces cerevisiae</u> mannan, <u>Aspergillus fumigatus</u> galactomannan, <u>alpha</u>-methylmannoside, N-acetylglucosamine and chitotriose. These properties pointed to a lectin-like receptor on the macrophage which bound the conidia.</p> <p>Mice with a thigh abscess due to <u>Candida albicans</u> have a heightened immune response to rabbit IgG anti-Candida antibody.</p> <p>In a large study of intravenous catheters used at the Clinical Center, culturing the tips of removed catheters did not aid in the diagnosis of catheter-acquired infection.</p> <p>The new allylamine class antifungal agent, SF 86-327, was found to give gradually increasing serum levels in patients given chronic therapy extending to one year.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00045-18 LCI
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>Studies on Interaction of Antibody and Complement on Production of Immune Damage</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Michael M. Frank, Chief, LCI/NIAID Others: Dr. Leonard Bielory, former Medical Staff Fellow, NIAID Dr. Chaim Brickman, former Medical Staff Fellow, NIAID Dr. Thomas Chused, Senior Investigator, LI/NIAID		
COOPERATING UNITS (if any) Dr. Thomas Lawley, NCI; Dr. Neil Young, NHLBI; Dr. George Tsokos, NIADDK; Dr. James Balow, NIADDK;		
LAB/BRANCH <u>Laboratory of Clinical Investigation</u>		
SECTION <u>Clinical Immunology Section</u>		
INSTITUTE AND LOCATION <u>National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892</u>		
TOTAL MAN-YEARS: <u>2.7</u>	PROFESSIONAL: <u>2.2</u>	OTHER: <u>0.5</u>
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.) <p>Prospective studies were performed of serum sickness in man. This was possible because of a NHLBI study of the treatment of aplastic anemia with horse antithymocyte globulin. Seventy per cent of treated patients developed serum sickness. In a prospective study it was shown that the onset of serum sickness in these patients could be correlated with the presence of circulating IgM containing immune complexes which bound Clq avidly. Positive RAJI assays were also noted although the amount of immune complexes noted in the latter assay was much lower than that noted by Clq binding. A new clinical finding was noted during the course of serum sickness in man and pathology of the GI system, kidney and joints was also noted in this disease. Prior to the onset of serum sickness histaminemia was noted in patients as well as an elevation in IgE levels. These studies show that in man, serum sickness follows much the same course as it does in experimental animals and validate the experimental animal model for studies of pathophysiology in man.</p> <p>Serologic findings associated with autoimmunity as well as clinical findings were studied in a series of 156 patients with Hereditary Angioedema. Although lupus was not a prominent finding in our patient population, a part or all of the symptom complex of Sjogren's Syndrome was a common finding in this patient group. It was noted that Clq binding activity was present in these patients. This was removable with a staph A column suggesting that IgG containing immune complexes were present in this patient group. An elevated number of helper T cells was present in the patient group as well, which was associated with decreased levels of C4 suggesting that complement activation leads to redistribution of T cells in these patients.</p>		

Other Professional Personnel

(name, title, laboratory, and institute affiliation)

T. E. Nash (Co-Principal Investigator)	Senior Investigator	LCI/LPD, NIAID
F. A. Neva (Co-Principal Investigator)	Senior Investigator/Chief	LCI/LPD, NIAID
R. Hussain	Staff Fellow/Visiting Fellow	LPD, NIAID
A. Cheever	Assistant Chief	LPD, NIAID
D. Keister	Biologist	LPD, NIAID
L. Miller	Head, Malaria Section	LPD, NIAID
M. Lunde	Research Zoologist	LPD, NIAID
T. Nutman	Medical Staff Fellow	LPD, NIAID
R. G. Crystal	Chief	PB, NHLBI
D. Sacks	Staff Fellow	LPD, NIAID
P. Scott	Staff Fellow	LPD, NIAID
B. Unger	Medical Staff Fellow	LPD, NIAID
D. Freedman	Medical Staff Fellow	LCI, NIAID
B. Curry	Visiting Associate	LPD, NIAID
J. Sherwood	Medical Staff Fellow	LPD, NIAID
D. Ward	Medical Staff Fellow	LPD, NIAID
R. Davey	Medical Staff Fellow	LCI, NIAID
R. Lal	Visiting Fellow	LPD, NIAID
A. Aggarwal	Visiting Fellow	LPD, NIAID
C. Lane	Senior Investigator	LIR, NIAID
W. Rom	Visiting Associate	PB, NHLBI
C. Chan	Senior Staff Fellow	CB, NEI
R. Nussenblatt	Chief	CB, NEI

Cooperating Units:

University of Arizona (E. Petersen); Peace Corps Medical Office, Washington, DC (K. Miller, M. Mulligan); Department of Health, Guatemala (G. Zea-Flores); University of Michigan (J. Bennett); University of Khartoum, Sudan (M. Homeida); Harvard University, Boston, MA (S. Ackerman, F. Von Lichtenberg); Onchocerciasis Chemotherapeutic Research Center, Tamale (K. Awadzi); Indian Council of Medical Research Tuberculosis Research Center, Madras, India (S.P. Tripathy, R. Prabhakar, P.R. Narayanan, V. Kumaraswami, R. Paranjape, and V. Vijayan); Special Programme for Tropical Disease Research, WHO, Geneva, Switzerland; Centers for Disease Control (C. Reimer); Madras Medical College, Madras, India (K. Vijayasekaran); Institute of Dermatology, Santa Domingo, Dominican Republic (H. Bogaert); Instituto de Salubridad y Enfermedades Tropicales, Mexico City, Mexico (O. Velasco); Rajendra Memorial Institute, Patna, India (L. Prasad); Walter Reed Army Institute of Research, Washington, DC (J. Chulay, W. Ballou); SEMA, Inc., Rockville MD (G. Phillips); Merck, Sharpe Dohme, Rahway, NJ (M. Aziz); and University of Maryland, Baltimore, MD (M. Levine).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00048-16 LCI
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) The Pathophysiology of Autoimmune Hemolytic Anemia		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Michael M. Frank, Chief, LCI/NIAID Others: T. Quinn, Senior Investigator, LIR/NIAID C. Hammer, Senior Investigator, LCI/NIAID L. Fries, Senior Staff Fellow, LCI/NIAID		
COOPERATING UNITS (if any) Dr. Jeffrey Moore, Hematology Branch, NHLBI; Dr. Neil Young, Hematology Branch, NHLBI; Dr. H.J. Müller-Eberhard, Scripps Clinic; B. Bender, Fellow in Infectious Disease, Johns Hopkins Hospital		
LAB/BRANCH Laboratory of Clinical Investigation		
SECTION Clinical Immunology Section		
INSTITUTE AND LOCATION National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 3.1	PROFESSIONAL: 2.5	OTHER: 0.6
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Studies of paroxysmal nocturnal hemoglobinuria led to the finding that two populations of erythrocytes are present in the patient group. One population is normal with respect to the membrane protein DAF (decay accelerating factor), while another population had no detectable protein. It was this population that lyses in PNH patients. It was shown that precursor cells which give rise to the DAF negative population and which are present in bone marrow in these patients, do not have the PNH phenotype. They do not lyse in acidified serum and have DAF on their surface. Thus, PNH was shown to be a developmental defect rather than a simple clonal abnormality as previously suspected.</p> <p>The nature of the C3 fragment leading to clearance of cells coated with cold agglutinin and incubated in serum were studied. Such cells were cleared by the liver rapidly when injected into the circulation. It was shown that such cells do not have the complement fragment C3b on their surface, but have C3bi on their surface. Thus for the first time in human beings it was shown that C3bi is capable of causing clearance in man.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00057-13 LCI
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Basic Studies on Pathogenic Fungi		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <div style="display: flex; justify-content: space-between;"> <div> PI: K.J. Kwon-Chung Other: W.L. Whelan </div> <div> Research Microbiologist Visiting Associate </div> <div> LCI, NIAID LCI, NIAID </div> </div>		
COOPERATING UNITS (if any) Department of Microbiology & Immunology, University of Southern Illinois, Springfield, IL (R. Tewari)		
LAB/BRANCH Laboratory of Clinical Investigation		
SECTION Clinical Mycology Section		
INSTITUTE AND LOCATION National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD		
TOTAL MAN-YEARS: 3.5	PROFESSIONAL: 2.0	OTHER: 1.5
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input type="checkbox"/> (b) Human tissues </div> <div> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Topics of current studies include: 1. virulence factors of <u>Cryptococcus neoformans</u>; 2. genetic complementation in <u>Cryptococcus neoformans</u>; 3. yeast-form growth and the viability of yeast cells of <u>Histoplasma capsulatum</u> in vitro; and 4. intragenetic effect of killer toxin produced by <u>Cryptococcus laurentii</u>.</p> <p>The relationship between encapsulation and phenoloxidase activity of <u>Cryptococcus neoformans</u> in the manifestation of virulence for mice was studied by employing the isolates with various combinations of Cap and Mel markers. The isolates with the <u>Cap⁺ Mel⁻</u> phenotype produced fatal infection in 90 to 100% of the animals within 40 days. The isolates with <u>Cap⁺ Mel⁺</u> phenotype produced fatal infection after 40 days, and 70 to 90% of the mice survived at least until day 70. Revertants, <u>Cap⁻ Mel⁻</u>, were recovered from the mice which died on day 68 after receiving an inoculum of <u>Cap⁺ Mel⁻</u> cells. The isolates with phenotypes of <u>Cap⁻ Mel⁺</u> and <u>Cap⁻ Mel⁻</u> not only failed to produce fatal infection but failed to revert to the <u>Cap⁺ Mel⁺</u> type in the mouse brain during the 70 day experimental period. A complementation test was devised for the fungus <u>Cryptococcus neoformans</u>. Complementation was signalled by the growth of prototrophic heterokaryons generated in crosses of the type aB X Ab, where a and b represent any two of the genetic auxotrophic markers. The complementing heterokaryons formed characteristic hyphal colonies. The viability of yeast form of <u>Histoplasma capsulatum</u> has previously been estimated by the use of dye exclusion test. Our study showed that the dye-exclusion test is unreliable for viability estimation and that counting of colony forming units produced on the <u>Histoplasma</u> growth factor medium was the only method which is reproducible as well as reliable. The isolates of <u>Cryptococcus neoformans</u> and <u>Cryptococcus albidus</u> were more sensitive to the killer toxin produced by an isolate of <u>Cryptococcus laurentii</u> than any other species of <u>Cryptococcus</u>.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00058-12 LCI
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Pathogenesis and Chemotherapy of Herpes Virus Infections in Man		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: S.E. Straus Senior Investigator, LCI, NIAID OTHER: J. Ostrove Senior Staff Fellow, LCI, NIAID J. Felser Medical Staff Fellow, LCI, NIAID K. Croen Medical Staff Fellow, LCI, NIAID M. Sawyer Medical Staff Fellow, LCI, NIAID A. Freifeld Medical Staff Fellow, LCI, NIAID J. Dale Clinical Research Nurse, LCI, NIAID		
COOPERATING UNITS (if any) J. Rooney Senior Staff Fellow, LOM, NIDR A. Notkins Chief, LOM, NIDR S. Nusinoff-Lehrman (Burroughs Wellcome Company)		
LAB/BRANCH Laboratory of Clinical Investigation		
SECTION Medical Virology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 3.0	PROFESSIONAL: 2.0	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The goals of this project are to characterize the natural history of mucocutaneous herpes simplex virus infections in normal and immune-impaired patients. In particular, we focus on genital herpes and the impact of brief and chronic acyclovir treatments on the natural history and expression of disease, and the <u>in vitro</u> sensitivity of virus recovered from patients. We have attempted to characterize the incidence of asymptomatic virus shedding from the genital area and to ascertain whether acyclovir alters those rates. The first convenient and efficient model of reactivation of herpes simplex virus infections in humans involving ultraviolet light exposure to previously infected skin sites has been established, and should serve to expand our comprehension of the temporal events and factors involved in disease expression.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00154-11 LCI																					
PERIOD COVERED October 1, 1985 to September 30, 1986																							
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Events in Immediate Hypersensitivity																							
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 40%;">PI: Michael A. Kaliner, M.D.</td> <td style="width: 40%;">Head, Allergic Diseases Section</td> <td style="width: 20%; text-align: right;">LCI/NIAID</td> </tr> <tr> <td>Others: Jay E. Slater, M.D.</td> <td>Medical Staff Fellow</td> <td style="text-align: right;">LCI/NIAID</td> </tr> <tr> <td>Martha V. White, M.D.</td> <td>NRSA Fellow</td> <td style="text-align: right;">LCI/NIAID</td> </tr> <tr> <td>Kaspar Sertl, M.D.</td> <td>Guest Researcher</td> <td style="text-align: right;">LCI/NIAID</td> </tr> <tr> <td>Ruth M. Jacobs, M.D.</td> <td>Medical Staff Fellow</td> <td style="text-align: right;">LCI/NIAID</td> </tr> <tr> <td>Mary Revenis, M.D.</td> <td>Guest Researcher</td> <td style="text-align: right;">LCI/NIAID</td> </tr> <tr> <td>Marek L. Kowalski, M.D.</td> <td>Fogarty Visiting Fellow</td> <td style="text-align: right;">LCI/NIAID</td> </tr> </table>			PI: Michael A. Kaliner, M.D.	Head, Allergic Diseases Section	LCI/NIAID	Others: Jay E. Slater, M.D.	Medical Staff Fellow	LCI/NIAID	Martha V. White, M.D.	NRSA Fellow	LCI/NIAID	Kaspar Sertl, M.D.	Guest Researcher	LCI/NIAID	Ruth M. Jacobs, M.D.	Medical Staff Fellow	LCI/NIAID	Mary Revenis, M.D.	Guest Researcher	LCI/NIAID	Marek L. Kowalski, M.D.	Fogarty Visiting Fellow	LCI/NIAID
PI: Michael A. Kaliner, M.D.	Head, Allergic Diseases Section	LCI/NIAID																					
Others: Jay E. Slater, M.D.	Medical Staff Fellow	LCI/NIAID																					
Martha V. White, M.D.	NRSA Fellow	LCI/NIAID																					
Kaspar Sertl, M.D.	Guest Researcher	LCI/NIAID																					
Ruth M. Jacobs, M.D.	Medical Staff Fellow	LCI/NIAID																					
Mary Revenis, M.D.	Guest Researcher	LCI/NIAID																					
Marek L. Kowalski, M.D.	Fogarty Visiting Fellow	LCI/NIAID																					
COOPERATING UNITS (if any) Marc M. Friedman, Ph.D., Georgetown University (Contract# NO-1-A1-22665)																							
LAB/BRANCH Laboratory of Clinical Investigation																							
SECTION Allergic Diseases Section																							
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205																							
TOTAL MAN-YEARS:	3.92	PROFESSIONAL: 1.75 OTHER: 2.17																					
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews																							
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Our analysis of events in immediate hypersensitivity focuses on human and animal models of allergic responses, mechanisms of mediator action, and pharmacologic approaches to allergic diseases. The areas under investigation include asthma, allergic rhinitis, anaphylaxis, urticaria, and mastocytosis.</p> <p>Employing monoclonal antibodies directed at cyclic GMP, the pattern of cells in guinea pig lung responding to histamine stimulation has been identified. Histamine causes alveolar cells, endothelial cells, epithelial cells, and macrophages to develop increased cyclic GMP. By contrast, atrionatriuretic factor causes smooth muscle cells to also increase their cyclic GMP. Ketotifen has been found to prevent histamine release from mast cells in patients with physical urticarias. Histamine levels in plasma are diagnostic of systemic mastocytosis if consistently elevated. The mechanism for progesterone-related anaphylaxis has been examined and remains unclear while a third progesterone-sensitive subject with anaphylaxis responded to LHRH analogue therapy. Microvascular permeability in skin is increased by histamine, serotonin, and bradykinin, but does not appear to contribute to the edema seen in late phase responses. Plasma histamine from patients in gram negative sepsis and shock is reduced below normal. BAL fluids obtained from asthma patients have normal histamine while interstitial pulmonary fibrosis is associated with elevated histamine. Neurogenic sensory stimulation leads to increased vascular permeability and mast cell degranulation. Human neutrophils release a factor which induces histamine release from RBL, human basophils, and guinea pig cutaneous mast cells. Rat lung has substance P receptors in the central airways which cause increased vascular permeability when stimulated.</p>																							

Other Professional Personnel (cont.):

Susan L. Wescott	Biologist	LCI/NIAID
Diane Bradley	Chemist	LCI/NIAID
Sandra M. Hurtado	Biologist	LCI/NIAID

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00155-11 LCI
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Phagocytic Cell Function		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: John I. Gallin, M.D. Scientific Director, IRP, NIAID Others: Harry L. Malech, M.D. Head, Bacterial Dis. Sect. LCI/NIAID Daniel Rotrosen, M.D. Medical Staff Fellow LCI/NIAID Judith Falloon, M.D. Medical Staff Fellow LCI/NIAID Philip Murphy, M.D. Medical Staff Fellow LCI/NIAID Christopher Brown, M.D. Medical Staff Fellow LCI/NIAID Joy Lennon, Ph.D. Visiting Associate LCI/NIAID COOP. UNITS: J. Nath, Ph.D., Dept. Hematol., Walter Reed Army Inst.		
COOPERATING UNITS (if any) Res., Washington, DC; B.E. Seligmann, Ph.D., Ciba Geigy, Summit, NJ; S.C. Dreskin, M.D., Ph.D., A&R, NIADDKD; C.J. White, M.D. IRP/NIAID; W. Zimmerli., M.D. Kantonsspital, Basel, Switzerland; Y. Ohno, M.D., Ph.D., Tenri Hosp., Japan;		
LAB/BRANCH Laboratory of Clinical Investigation		
SECTION Bacterial Diseases Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 4.7	PROFESSIONAL: 2.5	OTHER: 2.2
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The major objective of this project has been to study neutrophil function in health and disease. We have shown that a patient congenitally deficient in neutrophil specific granules has major abnormalities in neutrophil function. In normal neutrophils fmet-leu-phe receptors and C3bi receptors (CR3) are present in large amounts in an intracellular pool that cosediments with the specific granules from normal neutrophils on sucrose and Percoll gradients. These are translocated to the cell surface after activation of neutrophils resulting in a three fold increase in fmet-leu-phe receptor and CR3. CR3 deficient patients' neutrophils have abnormal chemotaxis, adhesiveness and spreading. Studies comparing peripheral blood and experimental exudate cells in guinea pigs and in man it has shown that there is preferential loss of neutrophil specific granules in the exudate cells. In addition, exudate cells have increased fmet-leu-phe and C3bi receptor expression. Cytochrome b, found in specific granules, is an important component of the electron transport chain essential for superoxide production. We have shown that neutrophil activation of the respiratory burst requires cytochrome b translocation from an intracellular compartment to the plasma membrane with activation by the calcium ionophore A23187 but not with activation by phorbol esters and fmet-leu-phe. Of 30 patients with chronic granulomatous disease, 11 had cytochrome b deficiency (10 were boys with an X-linked inheritance but interestingly one patient was a girl with probable autosomal recessive inheritance. In other studies of patients with hyper IgE syndrome (recurrent infections) we found that there is decrease catabolism of IgE in these patients.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00189-07 LCI
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Clinical and Biochemical Studies of Human Enteral Adenovirus Infections		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: S.E. Straus Senior Investigator, LCI, NIAID OTHER: J. Ostrove Senior Staff Fellow, LCI, NIAID		
COOPERATING UNITS (if any) H.S. Ginsberg, Columbia University, M. Levine, University of Maryland, G. Hammond, University of Manitoba		
LAB/BRANCH Laboratory of Clinical Investigation		
SECTION Medical Virology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 1.5	PROFESSIONAL: .25	OTHER: .25
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Enteroadenoviruses (EAd) comprise a serogroup with two related types of adenoviruses which are associated with gastroenteritis in infants. We have continued our studies of the epidemiology of enteric adenoviruses. We have recently subcloned a highly specific EAd DNA fragment which is being used in collaborative studies for rapid diagnosis of infection.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00192-08 LCI
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Studies on Immediate Hypersensitivity		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Dean D. Metcalfe, M.D. Head, Mast Cell Physiology Section LCI/NIAID Others: Daniel G. Malone, M.D. Medical Staff Fellow LCI/NIAID		
COOPERATING UNITS (if any) Arthritis and Rheumatism Branch, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases (R. Wilder), and The University of Washington (C.W. Henderson and S. Klebanoff)		
LAB/BRANCH Laboratory of Clinical Investigation		
SECTION Mast Cell Physiology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205		
TOTAL MAN-YEARS:	2.25	PROFESSIONAL:
		1.50
		OTHER:
		0.75
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.) <p>Rat mast cell heparin proteoglycan which has an estimated molecular weight of 750,000 is cleaved to molecules of <10,000 upon exposure to H₂O₂ and ferrous ion (Fenton's reagent). This cleavage is inhibited by catalase and by the hydroxyl radical scavenger mannitol. Heparin, as a component of intact mast cell granules, also is cleaved with H₂O₂ and Fe²⁺. These studies suggest that activated phagocytes which generate oxygen radicals contribute to the degradation of both solubilized and granule-associated proteoglycan heparin.</p> <p>Rat bone marrow-derived mucosal mast cells synthesize a chondroitin sulfate proteoglycan. Disaccharide analysis shows the predominant sulfated disaccharide to be chondroitin 4-sulfate.</p> <p>Mast cells were seen in 27 out of 35 synovial fluids. There was a strong correlation between mast cell number and histamine content. No relationship between mast cell number and diagnosis was observed except in patients with mastocytosis. Synovial fluid mast cells released histamine upon exposure to anti-human IgE, and contained a tryptase previously identified in human lung mast cells. In synovial biopsies from 20 patients with rheumatoid arthritis, mast cell infiltrations were shown to correlate with T helper/inducer lymphocytes. Mast cell numbers were dramatically decreased by intra-articular steroid injection, but not by methotrexate therapy.</p> <p>Endothelial cells in culture produce a factor which causes proliferation of the IL-3 dependent mast cell line PT-18.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00249-05 LCI
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) The Pathogenesis, Diagnosis, and Treatment of Systemic Mast Cell Disorders		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Dean D. Metcalfe, M.D. Head, Mast Cell Physiology Section LCI/NIAID Others: Robert B. Bressler, M.D. IPA Assignment LCI/NIAID		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Clinical Investigation		
SECTION Mast Cell Physiology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205		
TOTAL MAN-YEARS: <div style="text-align: center;">0.75</div>	PROFESSIONAL: <div style="text-align: center;">0.75</div>	OTHER:
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> We have previously shown that patients with systemic mastocytosis have elevated plasma histamine levels without the problems demonstrated in normal volunteers infused with histamine to a similar blood level. In an effort to determine how the body adapts to elevated histamine levels, we have examined end organ responses to histamine, and in the case of the skin, to the mast cell degranulator, codeine. It now appears that both the skin and lungs have normal dose response curves to histamine, and in the case of the skin, to codeine, when compared to normal subjects. Thus, patients with mastocytosis do not exhibit end organ desensitization to histamine, but adapt to increased histamine levels via an alternative mechanism. </p> <p> Cells with ultrastructural features of basophils may be observed in cultures of marrow from patients with mastocytosis. These cells are toluidine blue positive, contain histamine, and degranulate to the calcium ionophore A23187. Such cells increase in number under the influence of supernatants taken from cultures of human peripheral blood mononuclear cells stimulated with concanavalin A. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00250-05 LCI
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Clinical and Basic Studies on Inflammatory Diseases of the Gastrointestinal Tract		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Dean D. Metcalfe, M.D. Head, Mast Cell Physiology Section LCI/NIAID Others: Kim E. Barrett, Ph.D. Fogarty Visiting Fellow LCI/NIAID		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Clinical Investigation		
SECTION Mast Cell Physiology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205		
TOTAL MAN-YEARS: 0.85	PROFESSIONAL: 0.60	OTHER: 0.25
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) The issue of mast cell heterogeneity has been investigated in non-human primates by a comparative examination of lung and intestinal mast cells. These cells were obtained in parallel from the respective tissues of individual monkeys by an identical enzymatic dispersion technique. Mast cells derived from the lung differed from those derived from the intestine in that the majority of the former cell type could be stained with toluidine blue at pH 4-5, whereas the intestinal mast cells in the dispersed preparations required a more acidic pH (<1) to display metachromasia. In addition, the lung cells exhibited an increased content of the mast cell mediator histamine. Non-human primate lung mast cells were also quantitatively more responsive to an immunologic challenge than their intestinal counterparts in that they released a higher percentage of cellular histamine and generated more leukotriene C ₄ on stimulation. Considerable inter-animal variation was observed between the magnitude of mediator release from both mast cell types following anaphylactic activation, but evidence for the presence in non-human primates of the phenomenon of releasability was not obtained. The responsiveness of both cell types to a range of potential non-immunologic secretagogues and anti-allergic agents, including compound 48/80, substance P, theophylline and isoprenaline, was essentially similar. We conclude that mast cell heterogeneity in higher animals may be reflected more by cytochemical rather than by functional differences between mast cell classes.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00269-05 LCI
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Neutrophil Subpopulations		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: John I. Gallin, M.D. Scientific Director, IRP, NIAID Others: Harry L. Malech, M.D. Head, Bacterial Dis. Sect. LCI/NIAID Christopher C. Brown, M.D. Medical Staff Fellow LCI/NIAID Thomas Chused, M.D. Senior Staff LI/NIAID		
COOPERATING UNITS (if any) R. Jacobson, M.D. Georgetown Univ. Hosp., Dept. of Medicine Bruce E. Seligmann, Ph.D. Ciba-Geigy, Summit, New Jersey		
LAB/BRANCH Laboratory of Clinical Investigation		
SECTION Bacterial Diseases Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 1.48	PROFESSIONAL: 1.28	OTHER: 0.2
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Human <u>neutrophil heterogeneity</u> has been suggested on the basis of differences chemotactic responsiveness and on the basis of results in our laboratory showing differences in Fc receptor mediated rosetting of IgG coated red cells. Heterogeneity of functional responsiveness was examined using the <u>fluorescent activated cell sorter</u> to assess functional responsiveness of neutrophil populations stimulated with a chemoattractant normally produced by E. coli, <u>fmet-leu-phe</u> (fMLP). Heterogeneity of neutrophil binding of fMLP was shown. In the majority of neutrophils initial binding was to a high affinity <u>receptor</u> with subsequent appearance of a low affinity receptor that was readily displaced with additional fMLP. The cells with the two classes of receptors proved to be the ones that responded with depolarization of <u>membrane potential</u> and reduction of nitroblue tetrazolium dye in response to fMLP. The remainder cells (10-20%) only exhibited the high affinity receptor for fMLP and lacked evidence for activation by fmet-leu-phe. We developed an IgG <u>monoclonal antibody</u> (31D8) that binds strongly to neutrophils that are activated with fMLP was developed. The 31D8 antigen first appears at the myelocyte stage of maturation. We used 31D8 to study patients with <u>chronic myelogenous leukemia</u> (CML), a clonal disease. In 5 patients no 31D8 positive cells were detected. These 5 patients, who were in remission at time of initial study, subsequently progressed to accelerated phase or blast crisis. In CML absence of 31D8 antigen expression may be an early marker for progression of CML. Early detection would be useful since bone marrow transplantation appears to be most efficacious in CML when done early. We have also demonstrated that neonate cord blood <u>neutrophils</u> have a decreased number of 31D8 positive neutrophils.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00270-05 LCI
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>Tubulin Tyrosinolation in Normal and Abnormal Human Neutrophils</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Jayasree Nath, Ph.D. formerly Expert (left NIH) LCI/NIAID Currently Senior Investigator, Dept. Hematology, Walter Reed Army Institute of Research, Washington, DC Others: John I. Gallin, M.D. Scientific Director, IRP, NIAID		
COOPERATING UNITS (if any) Dr. Cynthia Oliver, Laboratory of Biochemistry, NHLBI, NIH		
LAB/BRANCH Laboratory of Clinical Investigation		
SECTION Bacterial Diseases Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 0.1	PROFESSIONAL: 0.1	OTHER:
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <u>PROJECT TERMINATED</u> Collaborative studies of tubulin tyrosinolation continue between Dr. Gallin and Dr. Nath within the context of Project #Z01 AI 00155-11, "Phagocytic Cell Function" with Dr. Gallin as PI. Tubulin tyrosinolation in neutrophils is stimulated by the chemotactic peptide fmet-leu-phe and the calcium ionophore A23187. This post-translational reaction requires calcium and activation of the NADPH oxidase. Neutrophil cytoplasts fail to demonstrate tubulin tyrosinolation in response to these stimuli indicating a requirement for intact neutrophils. Use of phorbol myristate acetate to activate neutrophils results in tyrosine incorporation into multiple proteins and is independent of protein synthesis. The phenomenon is dependent upon NADPH oxidase activation and is absent in neutrophils from patients with chronic granulomatous disease. Multi-protein tyrosinolation did occur in myeloperoxidase deficient neutrophils exposed to phorbol myristate acetate. The phorbol esters stimulate a two-fold increase in generation of protein carbonyl derivatives which is potentiated in the presence of labeled tyrosine. The phenomenon is specific for tyrosine as other amino acids like phenylalanine, leucine, histidine or methionine, fail to incorporate. The biochemical mechanism and the functional role of this intriguing reaction remains to be elucidated. However, the data suggest that tyrosine cross linking of peptides may be an important consequence of cell activation.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00271-05 LCI
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Purification and Characterization of Serum Complement Proteins and Fragments		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Carl H. Hammer, Senior Investigator, LCI/NIAID Others: Michael M. Frank, Chief, LCI/NIAID Louis Fries, Senior Staff Fellow, LCI/NIAID		
COOPERATING UNITS (if any) Thomas Lawley, DB/NCI; Kim Yancey, USUHS; Chaim Brickman, Sinai Hospital of Detroit; Lawrence Prograiss, Meharry Medical College		
LAB/BRANCH Laboratory of Clinical Investigation		
SECTION Clinical Immunology Section		
INSTITUTE AND LOCATION National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 1.8	PROFESSIONAL: 1.4	OTHER: 0.4
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Of critical importance to the function of the laboratory is the preparation of highly purified complement components with high specific activity. A new small scale procedure for the purification of C3 was developed which yields a fully active, homogeneous protein within three days with recovery of over 70%. We have also shown that C4, C5 and C9 as well are obtainable by this protocol. A rapid method for the purification of homogeneous fully active C1 Inhibitor has also been developed. Use of this inhibitor has allowed Drs. Tenner and Frank to demonstrate that on cell surfaces C4 prevents C1 Inhibitor from interacting with C1. C2 has been isolated by a recently developed, rapid, 3 step procedure involving PEG precipitation, DEAE-Sephacel chromatography and functional affinity chromatography on C4b-Sepharose. The method only needs quantitative analysis of recovery and functional purity for completion. A new rapid procedure for the purification of human C5a suitable for use in human subjects was developed. Extension of the new procedure has allowed for the isolation of C3a as well.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00272-05 LCI
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Host Defense Against Pneumococcal Bacteremia		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Richard J. Sveum, Medical Staff Fellow, LCI/NIAID Others: M.M. Frank, Chief, LCI/NIAID T. Chused, Senior Investigator, LI/NIAID		
COOPERATING UNITS (if any) E.J. Brown, Washington University School of Medicine, St. Louis, MO		
LAB/BRANCH Laboratory of Clinical Investigation		
SECTION Clinical Immunology Section		
INSTITUTE AND LOCATION National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 1.1	PROFESSIONAL: 1.1	OTHER: 0
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input type="checkbox"/> (b) Human tissues </div> <div> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.) <p>The project is presently concentrating on quantitative analysis of pneumococcal adherence and phagocytosis by human peripheral blood monocytes. A new method was developed that employs two fluorescent labels and dual laser flow cytometry that distinguishes between attached and ingested bacteria. The pneumococcal are covalently labeled with Lucifer Yellow which fluoresces green independent of pH. Surface bound pneumococci are labeled with biotinylated F(ab')₂ anti-capsular or anti-Lucifer antibodies to study opsonization requirements for adherence and rate of ingestion. We have also found that CR1 mediates binding and ingestion of serum opsonized pneumococci.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00273-04 LCI
PERIOD COVERED <u>October 1, 1985 to September 30, 1986</u>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>Role of Fibronectin in Opsonization and Phagocytosis</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Eric Brown	Senior Investigator LCI/NIAID
Others:	John Bohnsack, M.D.	Medical Staff Fellow LCI/NIAID
	George Martin, Ph.D.	Chief LDBA/NIDR
	Hynda Kleinman, Ph.D.	Senior Investigator LDBA/NIDR
	Gordon, Laurie, Ph.D.	Visiting Fellow LDBA/NIDR
COOPERATING UNITS (if any) Tsuneo Takahashi, American Red Cross		
LAB/BRANCH Laboratory of Clinical Investigation		
SECTION Clinical Immunology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) <div style="height: 300px; border: 1px solid black; margin-top: 10px;"></div>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00275-05
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) The Complement Receptor and C3 Mediated Opsonization		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Thelma Gaither, Research Biologist, LCI/NIAID Others: J. Gallin, Director, IRP, NIAID C. Hammer, Senior Investigator, LCI/NIAID M. Frank, Chief, LCI/NIAID		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Clinical Investigation		
SECTION Clinical Immunology Section		
INSTITUTE AND LOCATION National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 2.1	PROFESSIONAL: 1.1	OTHER: 1
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided) <p>The role of the PMN oxidative burst in Fc-mediated phagocytosis: Phagocytosis of selectively opsonized sheep erythrocytes (E) was studied. CGD and MPO deficient PMN were found to have markedly enhanced phagocytosis when compared with normal PMN. This enhanced activity apparently relates to deficiencies in oxidative burst in these cells. Phagocytosis by normal PMN was examined after treatment of the cells with agents known to block various steps of the oxidative burst. Sodium azide and cyanide, which block MPO, markedly enhanced phagocytosis by normal PMN. Scavengers of H₂O₂ (catalase), also enhanced phagocytosis, but not to the degree of sodium azide. Our studies indicate that the late acting products of the oxidative burst, produced by the interaction of the MPO-H₂O₂-Halide system, are primarily responsible for the abrogation of ingestion seen in normal PMN. Binding and ingestion of IgG, but not C3b-coated particles, appears to be regulated by this system.</p> <p>Complement receptor function in degradation of C3b: Neoplastic and normal human B cell lines were examined for their ability to degrade bound C3b. Raji cells, which do not express the C3b receptor, CR1, mediated C3b cleavage in the presence of the enzyme, Factor I. A series of studies was performed comparing the activity of Raji cells with cell lines derived from normal B cells and with human erythrocytes, which express only CR1. Inhibitors of Factor H (a known cofactor for I activity) and monoclonal anti-CR1 and anti CR2 (C3d receptor antibody) did not block the activity of the B cell lines. Anti CR1 completely blocked C3b cleavage by human E. This suggests that, in addition to CR1, other receptors or membrane factors on B cells participate in the physiologic degradation of C3b.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00276-05 LCI
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Studies of the Membrane Attack Complex of Human Complement		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Keith Joiner, M.D. Senior Investigator LCI/NIAID Others: Martin Sanders, M.D., Medical Staff Fellow, LCI/NIAID Michael M. Frank, M.D., Clinical Director, LCI/NIAID		
COOPERATING UNITS (if any) Dr. Moon Shin, University of Maryland, Baltimore, MD Dr. Lee Koski, University of Maryland, Baltimore, MD Dr. Elaine Alexander, Johns Hopkins University, Baltimore, MD		
LAB/BRANCH Laboratory of Clinical Investigation		
SECTION Clinical Immunology Section		
INSTITUTE AND LOCATION National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: .4	PROFESSIONAL: .4	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.) <p> Studies have continued on the demonstration of the terminal complement complex C5b-9 in fluids and tissues of patients with a variety of diseases. SC5b-9 was demonstrated in the serum of patients with Guillain Barre Syndrome (GBS) and chronic recurrent polyneuritis. The serum levels of SC5b-9 peaked on the 3-5th day after admission and declined thereafter, paralleling the levels of anti peripheral nerve myelin in serum as well as the disease status of the patients. C5b-9 was also detected by immuno histochemistry in the peripheral nerves from a patient with GBS. SC5b-9 was detected in the CSF from patients with Sjogren's Syndrome or Systemic Lupus Erythematosus who manifested CNS involvement. C5b-9 was not detected in patients without CNS involvement. C5b-9 was detected in the synovial membrane from patients with rheumatoid arthritis. Results from all of these studies suggest that generation of C5b-9 may contribute to the tissue damaging process in these diseases. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00277-05 LCI
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Mechanism of Serum Resistance in Bacteria		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Keith Joiner, M.D., Senior Investigator, LCI/NIAID Others: Michael M. Frank, M.D., Clinical Director, LCI/NIAID Carl Hammer, Ph.D., Senior Staff Fellow, LCI/NIAID		
COOPERATING UNITS (if any) Nili Grossman, LSB/NIADDK; Loretta Leive, LSB/NIADDK; John Foulds, LSB/NIADDK; Penny Hitchcock, LMSF/NIAID; Neal Schiller, U.C. Riverside; Martin Blaser, VA Med. Ctr., Denver, CO; Peter Rice, Boston Univ., Boston, MA; Ralph Judd, Univ. of Montana, Missoula, MT;		
LAB/BRANCH Laboratory of Clinical Investigation		
SECTION Clinical Immunology Section		
INSTITUTE AND LOCATION National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: .7	PROFESSIONAL: .7	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Studies were performed in three areas:</p> <p>1) Mechanism of serum resistance in gram negative bacteria. We found that C3 deposition on a serum resistant Salmonella strain occurred only on the small subset of LPS molecules bearing the longest O-Polysaccharide (O-PS) side chains, due to steric blocking of short O-PS side chains from complement attack. The LPS topography on the bacterial surface required to sterically block C3 deposition and to confer serum resistance was identified. Experiments on the mechanism of serum resistance in cystic fibrosis isolates of <i>Pseudomonas aeruginosa</i> and in <i>Campylobacter fetus</i> demonstrated that these organisms had unique mechanisms of serum resistance.</p> <p>2) Mechanism of bacterial killing by C5b-9. Experiments investigating release of periplasmic and cytoplasmic markers from <i>E. coli</i> J5 as the C9:C5b-8 ratio varied indicated that killing and release of the large periplasmic marker, beta-lactamase, and the small cytoplasmic marker, ⁸⁶Rb, required multimeric C9 within C9.</p> <p>3) Mechanism of action of bactericidal and blocking IgG for <i>Neisseria gonorrhoeae</i> (GC). Blocking IgG for GC competes with bactericidal IgG for binding, and leads to complement deposition at new sites on the outer membrane at which C5b-9 is not bactericidal. Bactericidal activity of monoclonal antibodies (Mab) to GC was also tested. A difference in the bactericidal activity of Mab to the major outer membrane (PI) of two strains bearing ostensibly identical PI's was due to minor amino acid differences in the surface exposed portion of the molecule. A Mab to the H8 antigen in GC, a recently described highly conserved antigen, was highly bactericidal for some but not all GC strains despite binding to the surface of all organisms.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00278-05 LCI
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Studies of the Components of the Complement Cascade		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Carl H. Hammer, Senior Investigator, LCI/NIAID Other: Michael M. Frank, Chief, LCI/NIAID (Study 1 and Study 3) Ronald G. Washburn, Medical Staff Fellow, LCI/NIAID (Study 2) John E. Bennett, Head, Clin. Mycology Sect., LCI/NIAID (Study 2)		
COOPERATING UNITS (if any) Hattie D. Gresham, Washington Univ., St. Louis (Study 1)		
LAB/BRANCH Laboratory of Clinical Investigation		
SECTION Clinical Immunology Section		
INSTITUTE AND LOCATION National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 1.9	PROFESSIONAL: 1.4	OTHER: 0.5
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <div style="margin-left: 40px;"> (Study 1) C5a generation <u>in vitro</u> by cell bound classical pathway convertase (EAC1423) was studied to determine whether there was any contribution to its expression by C6-9, the later acting complement components. It was shown that the presence of terminal components during activation of C5 allowed expression of markedly greater levels of C5a antigen and biological activity although consumption of C5 substrate was similar whether or not the terminal components were present. Free C5a antigen detected by radioimmunoassay correlated with C5a biological activity assessed by polymorphonuclear myeloperoxidase release. These findings describe a new role for the human terminal complement components in expression of biologically active C5a. </div> <div style="margin-left: 40px;"> (Study 2) <u>Aspergillus fumigatus</u> produces a water-soluble material which inhibits opsonization of fungal cells by normal human serum. This complement inhibitor (C.I.) was shown to decrease binding of the activated form of C3, C3b, to fungal surfaces by blocking alternative pathway activation. It appears to interfere with C3b deposition or activation. Since <u>A. flavus</u>, which is also a pathogen for humans produced this C.I. but <u>A. niger</u> did not, we speculate that <u>in vivo</u> production of C.I. may represent a pathogenesis factor for <u>Aspergillus</u> species by inhibition of opsonic, and chemotactic factors. We plan to purify this complement inhibitor to analyze its structure and using purified complement components determine its site of action in blockade of C3b deposition. </div>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00279-05 LCI
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Studies on Mucous Glycoproteins		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation) PI: Michael A. Kaliner, M.D. Head, Allergic Diseases Section LCI/NIAID		
COOPERATING UNITS (if any) James H. Shelhamer, M.D., Carolea Logun, and Jens D. Lundgren, Critical Care Medicine, Clinical Center		
LAB/BRANCH Laboratory of Clinical Investigation		
SECTION Allergic Diseases Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205		
TOTAL MAN-YEARS:	0.1	PROFESSIONAL: 0.1 OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) <p>Mucus secretion is a normal function of respiratory mucous membranes. Models for measurement of mucus production by cultured human and feline bronchial and nasal mucosae have been developed in order to examine the controls of mucus secretion. In addition to neurohormones and mediators of allergy, airways react to products generated by pulmonary macrophages and peripheral mononuclear cells with increased mucous glycoprotein secretion. The macrophage and mononuclear derived secretagogues are collectively being called macrophage/mononuclear cell derived mucus secretagogues (MMS).</p> <p>Corticosteroids inhibit MGP release by lowering baseline secretion. Analysis of corticosteroid treated airways reveals a close correlation between lipocortin generation and MGP production.</p> <p>Pulmonary inflammation with neutrophils is often associated with mucus production. Lysates of human neutrophils as well as supernatants from activated neutrophils cause airways to release MGP; this activity is not due to elastase, and identity of the mucus secretagogue is under study. Endorphins cause increased MGP secretion.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00354-04 LCI
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Immunoregulatory Defects in Inflammatory Bowel Disease		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Stephen P. James, M.D., Senior Investigator, Mucosal Immunity Section, LCI/NIAID Warren Strober, M.D., Head, Mucosal Immunity Section, LCI/NIAID Marjorie Kanof, M.D., NRSA Fellow, Mucosal Immunity Section, LCI/NIAID		
COOPERATING UNITS (if any) Claudio Fiocchi, M.D., The Cleveland Clinic Foundation, Cleveland, Ohio		
LAB/BRANCH Laboratory of Clinical Investigation		
SECTION Mucosal Immunity Section		
INSTITUTE AND LOCATION National Institute of Allergy and Infectious Diseases Building 10/11N250		
TOTAL MAN-YEARS: 2.5	PROFESSIONAL: 2.0	OTHER: 0.5
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> Crohn's disease is a chronic intestinal inflammatory disease of unknown etiology. One theory of pathogenesis is that it results from an abnormality of immunoregulation resulting in an unrestrained response to a ubiquitous intestinal antigen. We are testing this theory by analyzing T cell suppressor pathways in normal individuals and patients with Crohn's disease. </p> <p> Previous work from this laboratory has shown that the T cell population in lamina propria of the intestine differs from that in the peripheral blood in that the former population contains a much larger fraction of cells bearing a phenotype associated with helper-inducer function (Leu-3⁺8⁺) and a much smaller fraction of cells having the phenotype associated with suppressor-inducer function (Leu-3⁺8⁺) and suppressor-effector function (Leu-2⁺15⁺). In the present studies we examined antigen-non-specific immunoregulatory capacity of T cell populations derived from peripheral blood and lamina propria. The results indicate that peripheral blood Leu-3⁺8⁺ cells contain a Leu-3⁺8⁺ subpopulation with suppressor function. This is shown by the fact that when increasing numbers of Leu-3⁺8⁺ cells (which contain the Leu-3⁺8⁺ subpopulation) is cultured with normal B cells one obtains decreasing Ig synthesis. In addition, mixtures of Leu-3⁺8⁺ cells suppress indicator cultures containing Leu-3⁺8⁺ cells and B cells. A different picture is obtained for lamina propria T cells wherein one finds no decrease in Ig synthesis occurring with increasing numbers of Leu-3⁺8⁺ T cells. These findings are surprising in that they suggest that the Leu-3⁺8⁺ cells act, at least in part, on other Leu-3⁺8⁺ cells. In addition, they explain the fact that the lamina propria, in containing fewer Leu-3⁺8⁺ T cells than peripheral blood manifests less suppressor function. These results will be applied to the analysis of immunoregulatory cell function in Crohn's disease, in which case specific abnormalities of suppressor-inducer function will be sought. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00355-04 LCI
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Immunoregulatory Defects in Primary Biliary Cirrhosis		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Stephen P. James, M.D., Senior Investigator, Mucosal Immunity Section, LCI/NIAID Warren Strober, M.D., Head, Mucosal Immunity Section, LCI/NIAID		
COOPERATING UNITS (if any) E. Anthony Jones, M.D., Chief, Section on Liver Diseases, DOB/NIADDC		
LAB/BRANCH Laboratory of Clinical Investigation		
SECTION Mucosal Immunity Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD. 20892		
TOTAL MAN-YEARS 0	PROFESSIONAL: 0	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.) This project was not active during this fiscal year.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00356-04 LCI
PERIOD COVERED October 1, 1985 to September 30, 1985		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Studies of the Regulation of IgA Immunoglobulin Synthesis		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Warren Strober, M.D., Head, Mucosal Immunity Section, LCI, NIAID Michael C. Sneller, M.D., Staff Associate, MIS, LCI, NIAID Dennis Kunimoto, M.D., Fellow, Alberta Heritage Foundation, MIS, LCI, NIAID		
COOPERATING UNITS (if any) Fred Mushinski, M.D., Senior Investigator, Laboratory of Genetics, NCI, NIH Bethesda, MD. 20892		
LAB/BRANCH Laboratory of Clinical Investigation		
SECTION Mucosal Immunity Section		
INSTITUTE AND LOCATION Bethesda, Maryland 20892		
TOTAL MAN-YEARS: <div style="text-align: center;">3.0</div>	PROFESSIONAL: <div style="text-align: center;">2.5</div>	OTHER: <div style="text-align: center;">.5</div>
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard, unrounded type. Do not exceed the space provided.) <p> The aim of this project is to study, at the molecular level, the events involved in immunoglobulin Ig heavy chain C_H class switching as well as a mechanism by which T cells regulate such switching. In initial studies we sought a cellular system which would allow us to study B cell isotype differentiation employing clonal T cells and clonal B cell populations. Ultimately, we found that the B cell lymphoma 70Z/3 and the B cell lymphoma M12.4.1 can be induced to undergo isotype differentiation by a Peyer's patch-derived T cell hybridoma, HAJ 3. In studies of 70Z/3 b cells it was found that the 70Z/3 B cells expressed small amounts of membrane IgM (mIgM) and no membrane IgG (mIgG) prior to culture, whereas these B cells expressed large amounts of mIgGM and mIgG2b following co-culture with HAJ-3 T cells. Such new Ig expression is associated with the induction of IgG2b specific mRNA, but not with DNA switch region rearrangement or C_H deletion. The effect of LPS is quite different in that it stimulates 70Z/3 B cells to express considerable amounts of IgG2b mRNA, but does not induce expression of detectable mIgG2b; this indicates that a T cell influence is necessary for the production of translatable IgG2b mRNA. In studies of M12.4.1 cells we found that M12.4.1 B cells constitutively express mIgG2a, but upon co-culture with HAJ-3 T cells the mIgG2a disappears and mIgM and mIgG2b are induced. This induction is not associated with DNA recombination on deletion in that re-culture of induced M12.4.1 B cells in the absence of T cells leads to disappearance of mIgM and mIgG2b and then re-appearance upon re-cultured with HAJ-3 T cells. Finally, molecular analysis of T cell-induced M12.4.1 B cell expression of mIgM indicates that the latter is associated with nascent production of -specific mRNA synthesis. In all, these results are consistent with the idea that the first step of a B cell isotype differentiation is a reversible, T cell-induced step in which downstream gene transcription and translation is brought about in the absence of switch recombination and deletion. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00357-04 LCI
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Studies of the Autologous Mixed Lymphocyte Reaction		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
P.I.	Warren Strober, M.D.	Chief, Mucosal Immunity Section LCI, NIAID
OTHER:	Hiroyuki Kotani, M.D.	Visiting Scientist, MIS LCI, NIAID
	Stephen James, M.D.	Investigator, Mucosal Immunity Section LCI, NIAID
COOPERATING UNITS (if any) Hiroaki Mitsuya, M.D., Visiting Scientist, Clinical Oncology Program, NCI, NIH		
LAB/BRANCH Laboratory of Clinical Investigation		
SECTION Mucosal Immunity Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	1.3	0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> In this project we are studying the immunoregulatory potential of human T cells which are activated by self-MHC antigens present on B cells or macrophages (autoreactive T cells). In previous studies we established autoreactive T cell clones which are maintained in IL-2 enriched cultures by periodic stimulation with autologous non-T cells. One of these clones, termed MTC-4, was found to have dual immunoregulatory potential, providing help for resting B cells and suppression for pokeweed-mitogen (PWM)-activated B cells. In current studies we showed that MTC-4 helper function involves recognition of class II MHC antigens by the MTC-4 cells via its T3-associated T cell receptor. Further, we showed that the helper function is mediated by a BCGF-like factor which is distinct from IL-2 and which acts on both resting (small) and activated (large) B cells. Also in current studies we showed that MTC-4 T cell suppressor function is elicited when MTC-4 cells are co-cultured with non-T cells (B cells plus monocytes) that are pre-incubated with PWM for four hours, but not with non-T cells pre-incubated with PWM for 24 hours; this implies that induction of suppressor function depends on a transiently expressed MHC-associated surface molecule. In addition, we showed that pre-treatment of non-T cells with neuraminidase leads to non-T cells which do induce MTC-4 suppressor function. This suggests that the glycosylation status of non-T cells surface antigen determines whether non-T cells elicit helper or suppressor function from the autoreactive T cell population. Finally, we showed that the MTC-4 T cell suppressor function is MHC-specific in the effector phase in that only MHC matched cells are able to be suppressed. This indicates that MHC recognition is necessary in the effector cell-target cell interaction. In all, these studies establish that autoreactive T cells can be induced to provide either help or suppression depending on the type of B cell stimulus. This bifunctionality endows autoreactive cells with the potential for important homeostatic function. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00396-03 LCI
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Complement Receptors: Regulation of Expression and Cell Biology		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Alejandro Malbran, Visiting Fellow, LCI/NIAID Others: Louis Fries, Senior Staff Fellow, LCI/NIAID Michael M. Frank, Chief, LCI/NIAID		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Clinical Investigation		
SECTION Clinical Immunology Section		
INSTITUTE AND LOCATION National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 1.9	PROFESSIONAL: 1.4	OTHER: 0.5
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The objective of this work is to further define the physiologic mechanisms that regulate complement and immunoglobulin mediated phagocytosis. This laboratory has previously studied some of the cellular events that render a polymorphonuclear leukocyte or macrophage able to phagocytose via CR1, the receptor for the C3b fragment of the complement component C3. In resting neutrophils, this receptor does not mediate internalization or phagocytosis. However, when neutrophils are treated with phorbol esters, CR1 acquires two new activities; a) the ability to cause phagocytosis of C3b-coated particles, and b) a continuous and ligand-independent endocytic process. We have studied the fate of CR1 ligands and the receptor inside the cell following phorbol ester treatment. We have found that C3b CR1 complexes are recycled to the cell surface through a pre-lysosomal, pre-acidic compartment and that this compartment is associated with a low density membrane component. However, when CR1 is crosslinked, the recycling is slowed and diminished, and a different compartment of greater density containing the ligand is demonstrated. Future directions include the morphologic and kinetic characterization of this pathway in the cell, and its impact on phagocytosis mediated by a combination of Fc and CR1 receptors. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00397-03 LCI
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Interactions of C3b with Immunoglobulin G-Regulation of C3b Function by Antibody		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Louis F. Fries, Senior Staff Fellow, LCI/NIAID Others: Keith A. Joiner, Senior Investigator, LCI/NIAID		
COOPERATING UNITS (if any) Harvey M. Friedman, University of Pennsylvania; Gary H. Cohen, University of Pennsylvania; Roselyn J. Eisenberg, University of Pennsylvania		
LAB/BRANCH Laboratory of Clinical Investigation		
SECTION Clinical Immunology Section		
INSTITUTE AND LOCATION National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 1.0	PROFESSIONAL: 0.5	OTHER: 0.5
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) <p>Immunoglobulin G is known to enhance the rate and extent of alternative complement pathway activation on a variety of targets, and to facilitate complement-mediated destruction of some bacteria and virus-infected cells. We have shown that C3b residues covalently deposited on IgG are relatively protected from the actions of serum factors H and I, and hence have enhanced capacity to sustain alternative pathway activation. Human CR1 can overcome this protection, but binds weakly unless C3b is presented in a multivalent form. We have demonstrated that C3b-IgG complexes are highly efficient sensitizers of <i>E. coli</i> for complement-mediated killing and have suggested, on a statistical basis, the C3b bound to IgG may be the major C3b species mediating serum-killing of this organism. Future directions include studies of the mechanisms of enhanced complement lysis of C3b-IgG bearing targets, attempts to localize the C3b acceptor site on human IgG, and investigation of the opsonic capacity of C3b-IgG complexes.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00398-03 LCI																											
PERIOD COVERED October 1, 1985 to September 30, 1986																													
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Studies on the Interaction of Complement and Parasites																													
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 40%;">PI: Keith Joiner, M.D.</td> <td style="width: 40%;">Senior Investigator</td> <td style="width: 20%;">LCI/NIAID</td> </tr> <tr> <td>Others: Stephen Puentes</td> <td>Medical Staff Fellow</td> <td>LCI/NIAID</td> </tr> <tr> <td>Steven Fuhrman</td> <td>Medical Staff Fellow</td> <td>LCI/NIAID</td> </tr> <tr> <td>Maria Teresa Rimoldi</td> <td>Fogarty Fellow</td> <td>LCI/NIAID</td> </tr> <tr> <td>Andrea Tenner</td> <td>Visiting Fellow</td> <td>LCI/NIAID</td> </tr> <tr> <td>Carl Hammer</td> <td>Senior Staff Fellow</td> <td>LCI/NIAID</td> </tr> <tr> <td>Alan Sher</td> <td>Head, ICB Section</td> <td>LPD/NIAID</td> </tr> <tr> <td>David Sachs</td> <td>Senior Staff Fellow</td> <td>LPD/NIAID</td> </tr> <tr> <td>Bernis Dwyer</td> <td>Senior Investigator</td> <td>LPD/NIAID</td> </tr> </table>			PI: Keith Joiner, M.D.	Senior Investigator	LCI/NIAID	Others: Stephen Puentes	Medical Staff Fellow	LCI/NIAID	Steven Fuhrman	Medical Staff Fellow	LCI/NIAID	Maria Teresa Rimoldi	Fogarty Fellow	LCI/NIAID	Andrea Tenner	Visiting Fellow	LCI/NIAID	Carl Hammer	Senior Staff Fellow	LCI/NIAID	Alan Sher	Head, ICB Section	LPD/NIAID	David Sachs	Senior Staff Fellow	LPD/NIAID	Bernis Dwyer	Senior Investigator	LPD/NIAID
PI: Keith Joiner, M.D.	Senior Investigator	LCI/NIAID																											
Others: Stephen Puentes	Medical Staff Fellow	LCI/NIAID																											
Steven Fuhrman	Medical Staff Fellow	LCI/NIAID																											
Maria Teresa Rimoldi	Fogarty Fellow	LCI/NIAID																											
Andrea Tenner	Visiting Fellow	LCI/NIAID																											
Carl Hammer	Senior Staff Fellow	LCI/NIAID																											
Alan Sher	Head, ICB Section	LPD/NIAID																											
David Sachs	Senior Staff Fellow	LPD/NIAID																											
Bernis Dwyer	Senior Investigator	LPD/NIAID																											
COOPERATING UNITS (if any)																													
LAB/BRANCH Laboratory of Clinical Investigation																													
SECTION Clinical Immunology Section																													
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland																													
TOTAL MAN-YEARS: 3.4	PROFESSIONAL: 2.9	OTHER: .5																											
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews																													
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Epimastigotes of <u>T. cruzi</u> are lysed by the alternative complement pathway in normal human serum, whereas metacyclic trypomastigotes (CMT) and tissue culture trypomastigotes (TCT) resist lysis. 5-8 fold more C3 and C9 bind to Epi than to CMT or TCT duration incubation in NHS. We have shown that the major molecule to which C3 binds in Epi is a 72 Kd developmentally regulated glycoprotein, GP72, whereas the C3 acceptors in CMT are 40 Kd and 225 Kd molecules. The major form of C3 on Epi is C3b whereas the major form on CMT and TCT is hemolytically inactive iC3b. Inefficient alternative pathway activation by CMT is due to poor factor B binding to C3b on the parasite. Pronase rendered CMT sensitive to ACP lysis and resulted in enhanced B and C3 binding. CMT labelled by periodate oxidation and ³H Na BH₄ reduction showed a 90 Kd glycoprotein not labelled in Epi, a molecule which is specifically removed by pronase. Intrinsic labelling of CMT and TCT with ³⁵S methionine and subsequent incubation in buffer showed that this 90 Kd band was shed in almost pure form into the supernatant. Both CMT and TCT supernatant, as well as parasite lysates, contained components which were capable of inhibiting formation and accelerating decay of the alternative pathway C3 convertase. We are currently characterizing the molecules responsible for this decay accelerating activity.</p> <p>C3 binding was analyzed on promastigotes of <u>L. donovani</u>. The predominant form of C3 was iC3b. C3 bound to a parasite derived acid phosphatase by a hydroxylamine resistant (putative amide bond) but was rapidly released from the surface of the parasite by an unusual cleavage event. Similar release of C3 was found with non-infective, serum sensitive, log phase promastigotes of <u>L. major</u> but minimal release was detected from infective, serum resistant stationary phase <u>L. major</u>.</p> <p>Preliminary results show C3 binding to human erythrocytes infected with <u>Plasmodium falciparum</u>.</p>																													

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00428-02 LCI																
PERIOD COVERED October 1, 1985 to September 30, 1986																		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) IgE Immunotoxins																		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 45%;">Michael A. Kaliner, M.D.</td> <td style="width: 40%;">Head, Allergic Diseases Section</td> <td style="width: 10%;">LCI/NIAID</td> </tr> <tr> <td>Others:</td> <td>Howard Boltansky, M.D.</td> <td>Senior Staff Fellow</td> <td>LCI/NIAID</td> </tr> <tr> <td></td> <td>Jay E. Slater, M.D.</td> <td>Medical Staff Fellow</td> <td>LCI/NIAID</td> </tr> <tr> <td></td> <td>Sandra M. Hurtado</td> <td>Biologist</td> <td>LCI/NIAID</td> </tr> </table>			PI:	Michael A. Kaliner, M.D.	Head, Allergic Diseases Section	LCI/NIAID	Others:	Howard Boltansky, M.D.	Senior Staff Fellow	LCI/NIAID		Jay E. Slater, M.D.	Medical Staff Fellow	LCI/NIAID		Sandra M. Hurtado	Biologist	LCI/NIAID
PI:	Michael A. Kaliner, M.D.	Head, Allergic Diseases Section	LCI/NIAID															
Others:	Howard Boltansky, M.D.	Senior Staff Fellow	LCI/NIAID															
	Jay E. Slater, M.D.	Medical Staff Fellow	LCI/NIAID															
	Sandra M. Hurtado	Biologist	LCI/NIAID															
COOPERATING UNITS (if any) Richard Youle, Ph.D., National Institute of Neurological and Communicative Disorders and Stroke; and Otto Gansow, Ph.D., National Cancer Institute																		
LAB/BRANCH Laboratory of Clinical Investigation																		
SECTION Allergic Diseases Section																		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205																		
TOTAL MAN-YEARS:	1.9	PROFESSIONAL: 1.85 OTHER: 0.5																
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews																		
SUMMARY OF WORK (Use standard unrepduced type. Do not exceed the space provided.) Mast cells are the cellular nidus of allergic diseases and the cell responsible for disease in urticaria pigmentosa and systemic mastocytosis. This project is designed to ablate mast cells by attaching cytotoxic agents to IgE or antigen and selectively introducing the toxic product into mast cells. IgE linked to ricin kills RBL cells <u>in vitro</u> spontaneously or after cross-linking the IgE with anti-IgE. IgE linked to ricin's A chain kills in the presence of monensin, a carboxylic ionophore, or in the absence of monensin at higher concentrations. IgE-ricin A conjugates injected intradermally significantly lower rat cutaneous histamine levels <u>in vivo</u> suggesting that this agent might work <u>in vivo</u> as well.																		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00429-02 LCI
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Studies on Nasal Responses		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Michael A. Kaliner, M.D. Head, Allergic Diseases Section LCI/NIAID Others: Gordon D. Raphael, M.D. Medical Staff Fellow LCI/NIAID		
COOPERATING UNITS (If any) Robert F. Bonner, M.D., Biomedical Engineering and Instrumentation Branch, Division of Research Services; and James H. Shelhamer, M.D., Critical Care Medicine, Clinical Center		
LAB/BRANCH Laboratory of Clinical Investigation		
SECTION Allergic Diseases Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205		
TOTAL MAN-YEARS: <div style="text-align: right; margin-right: 20px;">1.1</div>	PROFESSIONAL: <div style="text-align: right; margin-right: 20px;">1.1</div>	OTHER: <div style="text-align: right; margin-right: 20px;">0</div>
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The Allergic Diseases Section began studying nasal provocation testing in 1982 when Dr. Howard Druce joined the section. Over the next 3 years, the laboratory focused on studies of nasal physiology with the plan to eventually study the allergic response in the nose. Dr. Robert Bonner developed the technique of laser-Doppler velocimetry (LDV) and, with Dr. Druce, adapted this technology to the study of nasal mucosal physiology. Using parameters including blood volume, blood flow, and pulsatility, baseline LDV measurements were recorded after saline nasal provocation, and then after provocation with methacholine, histamine, atropine, lidocaine, phenylephrine, and oxymetazoline. In addition to LDV measurements, nasal lavages were collected after nasal provocation, and these samples were assayed for total protein and albumin as a crude estimate of nasal secretion.</p> <p>Methacholine was found to stimulate protein secretion without an increase in albumin, indicating selective glandular secretion. Histamine stimulated albumin secretion (indicating increased vascular permeability) at low doses and both glandular secretion and vascular permeability at high doses. Atropine prevented methacholine effects and reduced histamine induced glandular secretion. Therefore, we can analyze the components of nasal protein secretion and determine their source.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00430-02 LCI
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Molecular Biology of Varicella-Zoster Virus Infections		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: S.E. Straus Senior Investigator, LCI, NIAID OTHER: J. Ostrove Senior Staff Fellow, LCI, NIAID J. Felser Medical Staff Fellow, LCI, NIAID K. Croen Medical Staff Fellow, LCI, NIAID M. Sawyer Medical Staff Fellow, LCI, NIAID A. Freifeld Medical Staff Fellow, LCI, NIAID G. Inchauspe Visiting Fellow, LCI, NIAID		
COOPERATING UNITS (if any) J. Hay (USUHS), W. Ruyechan (USUHS)		
LAB/BRANCH Laboratory of Clinical Investigation		
SECTION Medical Virology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 4.0	PROFESSIONAL: 3.0	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The major basic research focus of this laboratory involves this project, with the following goals:</p> <ol style="list-style-type: none"> 1) to identify, map and characterize varicella-zoster virus genes and proteins active in latent or productive infections. 2) to define the temporal sequence of gene expression. 3) to determine the interaction of antiviral drugs with viral gene products through a molecular analysis of drug-resistant mutants. 4) to characterize the molecular epidemiology of varicella-zoster virus infections. <p>To accomplish these ends we have constructed recombinant libraries of the complete VZV genome in a variety of vectors. The genome and most, if not all, of the major viral messages have been identified and preliminarily mapped, and two gene products representing immediate early and early functions have been identified and located. Our current efforts are directed at finer mapping of selected viral transcripts, defining the temporal sequence of gene expression using cell-free virus, and utilizing herpes simplex virus mutants to help identify and map complementary VZV gene functions in the establishment of an <u>in situ</u> hybridization system using a 35S riboprobe for detection of latent sequences within human trigeminal ganglia.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00432-02 LCI
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Regulation of Mucosal Immune Responses in Non-Human Primates		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Stephen P. James, M.D., Investigator, Mucosal Immunity Section, LCI, NIAID Martin Zeitz, M.D., Guest Researcher, Mucosal Immunity Section, LCI, NIAID		
COOPERATING UNITS (if any) Thomas Quinn, M.D., Investigator, Laboratory of Immunoregulation, NIAID		
LAB/BRANCH Laboratory of Clinical Investigation		
SECTION Mucosal Immunity Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD. 20892		
TOTAL MAN-YEARS: 2.0	PROFESSIONAL: 1.5	OTHER: 0.5
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The role of T cells in inflammation in the GI mucosa was studied in Chlamydia trachomatis (LGV biovar) proctitis in non-human primates. LGV-induced proliferative responses were found in lymphocytes from peripheral blood, spleen, mesenteric and local draining lymph nodes. Using dual laser FACS and cell separation techniques it was shown that both CD4⁺ and CD8⁺ T cells proliferate and that B cells have a T cell-dependent proliferative response to LGV antigens. In contrast, isolated lamina propria T cells failed to demonstrate proliferative responses to LGV, which was not due to cell mediated suppression or lack of antigen-presenting cells. Nonetheless lamina propria T cells had the capacity to provide help for IgG synthesis in response to LGV. In cyclosporin treated animals, the expected suppression of peripheral T cell responses was found, but there was a paradoxical increase in proliferation of gut-associated lymphocytes. These findings suggest that the efferent limb of the mucosal immune system is adapted to expansion of both helper and suppressor T cell pathways, but in the intestinal mucosa T cells primarily carry out effector functions such as providing help for antibody synthesis. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00447-02 LCI
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Clq: Its Biosynthesis and Biological Functions		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: David Bobak, Medical Staff Fellow, LCI/NIAID Others: Michael M. Frank, Chief, LCI/NIAID Andrea Tenner, Senior Staff Fellow, LCI/NIAID		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Clinical Investigation		
SECTION Clinical Immunology Section		
INSTITUTE AND LOCATION National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 1.9	PROFESSIONAL: 1.8	OTHER: 0.1
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) <p>The goals of this project are twofold: 1) the characterization of ClqR expression on human polymorphonuclear phagocytes (PMN) and monocytes, and 2) investigation into the modulation of IgG- and complement-mediated phagocytosis by Clq.</p> <p>Clq is a ligand for cell surface receptors on a variety of cell types. In the last year we have further characterized the Clq ligand-receptor interaction on human PMN and monocytes. We have shown that the chemoattractant n-formylleucylphenylalanine (fMLP), and the phorbol ester phorbol dibutyrate (PDBu) do not upregulate ClqR number on PMN and monocytes. This is in contrast to the behavior of other complement receptors (CR1, the C3b receptor; CR3, the iC3b receptor) presented with the same stimuli. We are presently investigating the possibility that the ClqR is internalized during response to fMLP and PDBu.</p> <p>In the last year, we have also found that solid phase Clq can greatly enhance Fc receptor mediated phagocytosis in human monocytes and culture-derived macrophages. This effect is dose responsive, can be blocked by anti-Clq, and seems to be mediated via the collagen-like tail region of the Clq molecule; the same region of the molecule known to interact with the ClqR. Present studies involve the further characterization of this response and its possible relation to that effect seen with the extracellular matrix proteins laminin and fibronectin.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00469-01 LCI
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Complement: Studies in Viral Infection		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Louis F. Fries, Senior Staff Fellow, LCI/NIAID Others: Keith A. Joiner, Senior Investigator, LCI/NIAID		
COOPERATING UNITS (if any) Harvey M. Friedman, University of Pennsylvania; Gary H. Cohen, University of Pennsylvania; Roselyn J. Eisenberg, University of Pennsylvania		
LAB/BRANCH Laboratory of Clinical Investigation		
SECTION Clinical Immunology Section		
INSTITUTE AND LOCATION National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 1.0	PROFESSIONAL: 0.6	OTHER: 0.4
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> Infection with herpes simplex type 1 induces expression of receptors for C3b on mammalian cells previously devoid of such receptors. We have shown that the isolated glycoprotein responsible for this activity (gC) is a potent modulator of complement-mediated cytolysis in-vitro, acting by at least two mechanisms. We have also aided in the demonstration of relative complement resistance by cells infected with wild type HSV-1, as opposed to cells infected with gC-defective mutant strains. Future plans include direct binding studies of purified C3 fragments to HSV-1 gC and defined fragments thereof, as well as cells infected with other human herpesviruses. The role of gC as a blocker of infected cell interactions with cytotoxic lymphocytes will also be studied. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00470-01 LCI		
PERIOD COVERED October 1, 1985 to September 30, 1986				
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.) A Study of Sporadic Neurasthenia Associated with Epstein-Barr Virus				
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 35%; vertical-align: top;"> PI: S.E. Straus OTHER: J. Dale J. Ostrove K. Croen </td> <td style="width: 65%; vertical-align: top;"> Senior Investigator, LCI, NIAID Clinical Research Nurse, LCI, NIAID Senior Staff Fellow, LCI, NIAID Medical Staff Fellow, LCI, NIAID </td> </tr> </table>			PI: S.E. Straus OTHER: J. Dale J. Ostrove K. Croen	Senior Investigator, LCI, NIAID Clinical Research Nurse, LCI, NIAID Senior Staff Fellow, LCI, NIAID Medical Staff Fellow, LCI, NIAID
PI: S.E. Straus OTHER: J. Dale J. Ostrove K. Croen	Senior Investigator, LCI, NIAID Clinical Research Nurse, LCI, NIAID Senior Staff Fellow, LCI, NIAID Medical Staff Fellow, LCI, NIAID			
COOPERATING UNITS (if any) M. Kruesi (LCS, NIMH), G. Tosato (BOB, FDA), G. Armstrong (BOB, FDA), O. Preble (USUHS), G. Pearson (Georgetown Univ.), W. Henle (Children's Hosp., Philadelphia)				
LAB/BRANCH Laboratory of Clinical Investigation				
SECTION Medical Virology Section				
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892				
TOTAL MAN-YEARS: 1.5	PROFESSIONAL: 1.25	OTHER: 0.25		
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews				
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The goal of this project is to characterize a chronic fatigue syndrome and to ascertain whether Epstein-Barr virus plays a role in its causation. We have identified and are further exploring a series of clinical and immunologic abnormalities in some patients. We have nearly completed a large placebo controlled trial of acyclovir therapy in 27 patients with unusual Epstein-Barr virus serologic profiles. Our desire is to determine whether chemotherapy alters symptoms, virus shedding rates or any of a series of immunologic abnormalities we have observed. Our planned studies in this area involve a heightened focus on individuals whose illness began with well documented acute infectious mononucleosis and on the molecular properties of virus recovered from them.</p>				

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00481-01 LCI
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Receptors and Transduction Mechanisms in Human Phagocytes		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Harry L. Malech, M.D. Head, Bacterial Dis. Sect. LCI/NIAID Others: John I. Gallin, M.D. Scientific Director, IRP, NIAID Daniel Rotrosen, M.D. Medical Staff Fellow LCI/NIAID Judith Falloon, M.D. Medical Staff Fellow LCI/NIAID Philip Murphy, M.D. Medical Staff Fellow LCI/NIAID		
Coop. Units: Allen Speigel, MDB, NIADDKD; Marc M. Friedman, Ph.D, Molec. Virol. Immunol. Div., Georgetown University,		
COOPERATING UNITS (if any) Rockville, MD.; Edward J. Benz, Jr., M.D., Ph.D., Sec. Hematol., Dept. Medicine, Yale University, New Haven, CT.; Donald F. Heiman, M.D., Sect. Infect. Dis., Dept. Medicine, Chicago Medical School, North Chicago, IL.		
LAB/BRANCH Laboratory of Clinical Investigation		
SECTION Bacterial Diseases Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 1.7	PROFESSIONAL: 1.1	OTHER: 0.6
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Human neutrophils and monocytes are attracted to sites of inflammation and engulf invasive microorganisms or host cell debris. These phagocyte responses are mediated by surface receptors for a variety of substances released by infective agents or host, or derived from host plasma. The purpose of this project is to define the biochemical structure and transduction mechanisms of some of these surface receptors. More specifically, the present studies focus upon: (i) the receptor for bacterially derived formyl peptides, the formyl peptide chemotactic receptor (FPCR); (ii) the receptor for the particle-adherent C3 complement fragment iC3b (CR3); (iii) the pertussis toxin inhibitable GTP binding regulatory protein (Gi) in neutrophils required for FPCR and other receptor mediated cellular responses. Using affinity labeling of FPCR in conjunction with enzymatic cleavage or metabolic inhibitors we have shown that this receptor is highly N-glycosylated containing both complex- and high mannose-type polysaccharides. The polypeptide portion of FPCR appears to be of similar molecular weight with a similar proteolytic cleavage pattern in different phagocytic cells. N-glycosylation of FPCR is not required for ligand binding or stimulus-response coupling to occur. Using antibodies directed at different GTP regulatory proteins, we have shown that the Gi in neutrophils is immunologically distinct from the homologous protein in brain. Using immunogold labeling of CR3 we have shown by electron microscopy that average density of surface CR3 doubles after stimulation with phorbol myristate acetate and that these receptors are clustered on the cell surface. This arrangement may be important for the neutrophil adherence mediated by this receptor.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00482-01 LCI
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>Endothelial Cells in Inflammation</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Daniel Rotrosen, M.D. Medical Staff Fellow LCI/NIAID Others: John I. Gallin, M.D. Scientific Director, IRP, NIAID Harry L. Malech, M.D. Head, Bacterial Dis. Sect. LCI/NIAID		
COOPERATING UNITS (if any) Kevin Foskett, Ph.D., Department of Physiology, Armed Forces Radiobiology Research Institute, Bethesda, MD		
LAB/BRANCH Laboratory of Clinical Investigation		
SECTION Bacterial Diseases Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 0.84	PROFESSIONAL: 0.84	OTHER: ---
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The vascular <u>endothelial cell</u> is uniquely situated to play an active role in the transduction of pro-inflammatory signals from the interstitial tissues to circulating <u>neutrophils</u>. The goals of our principal projects are (i) to study the role of endothelial <u>cytosolic calcium</u> levels in alterations in vascular permeability in response to humoral inflammatory mediators, and (ii) to examine the role of the endothelial cell in transduction of <u>chemoattractant</u> signals from interstitial sites to circulating leukocytes. Using the fluorescent calcium indicator <u>quin2</u>, we have shown a rise in endothelial cytosolic calcium in response to the neutrophil independent promoters of vascular permeability (<u>histamine</u> and acetyl glyceryl ether phosphoryl choline [platelet activating factor]) whereas neutrophil dependent promoters of increased vascular permeability (<u>C5a</u>, <u>formyl-methionyl-leucyl-phenylalanine</u>, and <u>LTB₄</u>) elicit no change in endothelial cytosolic calcium. We have characterized in detail the alterations in endothelial cytosolic calcium in response to histamine and correlated these with changes in endothelial shape and monolayer integrity monitored by macromolecular diffusion across cultured endothelial monolayers. </p> <p> Recent reports have demonstrated receptor mediated endocytosis and vectorial transport of intact insulin by cultured vascular endothelial cells. We have confirmed a prior observation that endothelial cells bind formyl peptide chemoattractants and are investigating the possibility that, in an analogous fashion, the endothelial cell directs transport of chemoattractant peptides from interstitial sites to the vascular lumen. </p>		





LABORATORY OF IMMUNOGENETICS
1986 Annual Report
Table of Contents

<u>Z01-AI</u> <u>Project Number</u>		<u>Page</u>
Summary Report		4-1
00166-09	Characterization of Rabbit MHC Antigens -- Kindt	4-9
00168-09	Cell Surface Markers of Rabbit Lymphocytes -- Kindt	4-10
00169-09	Analysis of Murine and Human Transplantation Antigens and Genes -- Coligan	4-11
00170-09	Studies of Human Histocompatibility Antigens -- Long	4-12
00171-09	Genetic Studies of Rabbit Immunoglobulins and Other Serum Proteins -- Sogn	4-13
00173-09	Control of Gene Expression -- Max	4-14
00180-08	Properties of Transformed Rabbit Cell Lines -- Sogn	4-15
00352-04	Characterization of Cell Surface Molecules Important for Immune Function -- Coligan	4-16
00389-03	Molecular Genotype Analyses in HLA Recombinant Human Families -- Robinson	4-17



Annual Report
Laboratory of Immunogenetics
National Institute of Allergy and Infectious Diseases
October 1, 1985 to September 30, 1986

RESEARCH PROGRESS

The Laboratory of Immunogenetics investigates the multigene families that are involved in the control of immune function. Research emphasizes the structure and function of these genes and their products as well as mechanisms for regulation of these genes. Recent investigations have emphasized the major histocompatibility complex, the T cell antigen receptor and the immunoglobulin gene complexes of several different species. A wide range of techniques at the molecular, serological and functional level are used in these investigations.

STUDIES OF THE MAJOR HISTOCOMPATIBILITY COMPLEX

Investigations of major histocompatibility complex genes include studies of the structure and function of genes and products of the human class I and class II loci, structural studies of the murine class I system aimed at defining the diversity of this system as well as studies of the rabbit class I and class II genes and their expression in various cells and tissues.

Human class II MHC antigens. The human immune response genes are located in the D region of the human MHC. These include the α and β chains of the HLA-DR, -DQ and -DP class II antigens. It has been attempted by a molecular approach to determine how many functional class II genes exist and to express the individual class II antigens in cell lines by DNA mediated gene transfer in order to analyze the interaction between the class II antigens and the T lymphocytes. A new class II gene was identified in this study and was characterized as a complete cDNA clone (Long). This gene is called DO β . Another α (DZ α) chain has recently been described. These new genes do not however comprise an $\alpha\beta$ pair because of differences in the expression pattern. DZ α is more like a typical class II gene in that it is found in B cells, activated T cells and cells treated with γ interferon (Rosen-Bronson). DO β , on the other hand, is expressed only in B cells.

The cDNA clones for the various class II antigens have been placed in eukaryotic expression vectors and transfected into various murine, simian and human cell lines which express defined class II antigens at their surface (Sekaly). This expression system has allowed demonstration that the invariant chain, a glycoprotein associated intracellularly with class II antigens, is not necessary for the cell surface expression of the class II antigens. It has also been possible to show that DR antigens interact with the T4 molecule, a cell surface antigen expressed by a subset of T lymphocytes. Transfected cell lines expressing class II antigens are able to present foreign viral antigens to T lymphocytes with appropriate specificity for the viral antigen and the MHC molecules. This system may now be used to define elements important for antigen recognition by T lymphocytes.

The availability of the new genes in the HLA-D region have allowed a linkage study of class II genes at the molecular level. This study utilized the technique of pulsed-field gel electrophoresis and allowed mapping of the D β and the D α genes within the 1100 kilobases present in the D region. The availability of full length clones D β and D α make it possible to analyze these new genes at the protein level.

Variation in human and murine transplantation antigens. Human and murine major histocompatibility complex encoded molecules have been studied in an effort to gain a molecular understanding of their functional and antigenic properties. A variant of the human class I antigen HLA-A3 that is altered in its ability to present influenza antigens has been studied by a direct sequence analysis (Cowan). These studies reveal that the variation in this antigen most likely derives from a gene conversion event. Studies utilizing mouse class I antigens have determined that class I molecules in the H-2 system achieve additional diversity through the use of alternative transcripts of the same gene (Coligan). Alternative forms of certain class I molecules appear to be differentially expressed in T and B lymphocytes suggesting they may have some functional role. Factors controlling the expression of the murine soluble class I antigen (Q10) have been investigated and it was shown that different haplotypes may vary greatly in the level of this product (Lew).

Additional studies in the mouse have revealed a second family of D region molecules which is present in several distinct haplotypes. This family designated Dw3 is distinct from the previously defined L^L family of molecules (Lillehoj).

Studies of rabbit major histocompatibility complex. The class I and class II gene families in the MHC of the rabbit are being investigated by molecular techniques. The class I gene family was found to contain approximately 10 genes falling into four subfamilies. Structural data are available for four rabbit cDNA clones and the genomic clones corresponding to three of these have been completely or partially characterized (Marche).

One of the genes in the class I family corresponds to a cDNA clone designated pR27. A probe may be derived from this clone that is specific for a single copy MHC gene. Use of this probe to study gene expression has revealed that pR27 is expressed only in the rabbit T cell line RL-5 and in mRNA derived from rabbit thymus. The transcript for the pR27 gene is larger than the normal rabbit class I transcript. Studies are presently under way using S1 nuclease mapping to determine the precise splicing pattern in RNA derived from RL-5 and normal thymus (Rebiere). This technique can detect low levels of expression of this gene in normal tissues and determine splicing patterns for the larger than normal transcripts. The pR27 cDNA contains at its 3' terminus approximately 350 nucleotides which when used as probe behave as a repetitive sequence. A homology study of this region indicates a very strong homology to a region in the rabbit protein uteroglobulin. The significance of this homology (approximately 80% for a stretch of 230 bp) is currently being investigated.

Studies of rabbit class II genes indicate that the rabbit class II family contains approximately five α genes which have been designated RLA-DR, -DQ, -DP1, -DP2 and -DN (LeGuern). Probes specific for each of these have been isolated and have been used in surveys for mRNA expression

in normal and pathological tissues of the rabbit as well as in various cell lines that have been derived from this species (Kulaga). The results are for the most part consistent with assignment of these genes as homologues of the rabbit α genes by strength of hybridization and by sequence homology. The RLA-DR, -DQ and -DP1 α genes are expressed in lymphoid tissue and in cells activated with γ interferon. Expression studies of the rabbit DP2 α gene have shown that a larger (3.6 kb) transcript that has expression patterns not unlike that of the gene HLA-DZ. Sequence analogies are being carried out to determine the precise relationship of these genes. Studies on RLA-DN α have indicated that expression of a large transcript may be observed most strongly in heart tissue. This phenomenon is under study. Sequence analysis of the RLA-DN clone has revealed poor homology to class II genes with the exception of a small stretch corresponding to intron 4 and exon 4 of HLA-DR α .

A number of class II positive rabbit continuous cell lines have been established by in vitro viral transformation (Kulaga). The properties of these cell lines have been studied and most are found to have properties consistent with identification as macrophages. Preliminary evidence indicates that use of HTLV-1 will allow transformation of rabbit T lymphocytes.

The sequence of rabbit gene corresponding to human HLA-DQ has been completed both for cDNA and a genomic clone (Bagnato). These studies indicate a very strong sequence homology between the human and rabbit counterparts.

Class I MHC antigens of the hamster. The oncogenic adenovirus 12 appears to eliminate class I antigen expression in transformed cells in order to escape host detection. The non-oncogenic adenoviruses 2 and 5 have no such effect. A study of the class I antigen expression in the hamster which readily accepts the adenovirus infection is now underway (Sogn). Because the hamster MHC class I locus is poorly defined it is necessary to study basic elements of this gene family. The class I gene complex in the hamster is now under investigation and a number of class I genes cloned from a genomic library have been mapped. The identity of expressed cell surface antigens will be determined by examination of cDNA clones in this study.

STUDIES OF THE T CELL ANTIGEN RECEPTOR GENES

The T cell antigen receptor has been studied in the human, the rabbit, the mouse and the guinea pig. Polymorphisms have been identified for the α and β chain genes of this receptor. Studies in progress will determine whether polymorphisms will allow functional distinction of the genetically divergent individuals.

T cell polymorphism in man. In previous studies, polymorphic restriction fragments hybridizing with a T cell receptor β chain probe were observed to segregate in eight families studied. Haplotype assignments could be made in some families but it was not possible for certain of the others. Two additional polymorphisms have been observed with a probe corresponding to a variable gene segment in the T cell β complex and these have been used to complete assignment of haplotypes for nearly all of the individuals studied (Robinson). Different combinations of the constant (C)

and two variable (V) region markers result in eight possible distinct haplotypes. All but one of these haplotypes was observed in the parents of the families studied. The data regarding segregation of the polymorphic V and C fragments in families provide support for linkage of the V and C region segments but the diversity of haplotypes at the population level argues that recombination has occurred between the V and C region gene segments and among members of the V region subfamilies marked by the probes used.

Studies of the T cell receptor α chain have been carried out using a variable region probe derived from the studies of the rabbit and human probe that includes constant and 3' untranslated regions. The T cell α haplotypes are quite polymorphic and these polymorphisms should provide useful markers that will facilitate linkage studies, mapping studies, and genetic analysis of T cell function. These markers can provide a means of evaluating the role of T cell receptor genes in disease susceptibility in populations.

Collaborative studies are presently under way to assess the possible role of different T cell receptor genotypes in various disease states. Preliminary results from another laboratory have shown that the susceptibility to insulin-dependent diabetes mellitus is significantly increased in individuals with certain T cell receptor types. Studies of other diseases that have shown increased relative risk for individuals with different HLA haplotypes will be studied in order to determine whether the relative risk factor increases or decreases in persons with different T cell receptor haplotypes. In addition, functional studies of individuals with cellular reactivities inconsistent with HLA types will be studied in order to determine what influence the T cell receptor genes have on such aberrant reactivity.

Polymorphisms in T cell receptor genes have been observed in rabbit, pig, and in certain wild mouse strains (Kindt). These studies indicate that the restriction fragment length polymorphism present in the T cell receptor complexes can provide useful markers for studies both of evolutionary history of the animal in question, as well as for functional studies involving immune reactivity at various levels.

T cell antigen receptor in the rabbit. The rabbit T cell line, RL-5, has been used for studies concerning the T cell receptor genes in the rabbit (Marche). Probes corresponding to the constant region of the murine T cell receptor α and β chains were used to screen genomic and cDNA libraries constructed from this line. Two full length α transcripts were isolated and their sequences determined. One of the transcripts contained a single base pair deletion in the variable region which placed the remainder of the transcript out-of phase. The other transcript encoded a complete α chain corresponding to leader variable region, J region, constant and 3' untranslated regions.

The observation of two full length α transcripts in a cloned cell line suggests that the mechanism for allelic exclusion in the T cell receptor bearing cells operates at a level beyond transcription of the rearranged gene. The only defect in the transcript was a single base deletion that was not evident until the sequence analysis had been carried out.

Studies of the β chain transcripts produced in RL-5 indicated that there are two transcripts: one was 1.3 kb in length and the other approximately 1 kb in length. Sequence analysis of these indicated that the larger corresponds to a complete T cell β chain whereas the other is an incomplete transcript lacking a variable region. Probes derived from the β chain variable region revealed a multigene family in rabbit, pig and man, however, this probe used at the same stringency detected no bands in DNA samples from mouse, rat and hamster. A human gene from the cell line Molt 4 has approximately 75% nucleic homology to the β chain variable region that was isolated from RL-5. By contrast, of all of the murine genes that have been sequenced (approximately 30) only one, V β 15, has any significant homology to the rabbit gene and this is about 64% nucleic acid homology.

Comparisons of the constant region of the rabbit β chain genes to those of man and mouse indicate an extremely high degree of conservation within species for the β 1 and β 2 genes. There is a significant but considerably lower conservation among the β genes of the different species studied. When the 3' untranslated regions of the β genes are compared however, some conservation of C β 1 and C β 2 specific sequence is evident from the comparison. These data suggest that there is some mechanism which allows correction of the C β 1 and C β 2 coding region genes in a given species.

CONTROL OF GENE EXPRESSION

Mechanisms of gene expression control are being investigated for genes that are active in human B lymphocytes. The specific genes under study are immunoglobulin κ light chain (Gimble) and the J chain gene. High resolution electrophoretic techniques originally developed for genomic sequence analyses have been used to examine the accessibility of proposed enhancer regions, endonucleases and to dimethylsulfate. This so-called *in vivo* footprint analysis revealed a 2.5 kb segment of DNAase-I hypersensitivity region which is also accessible to restriction endonucleases. This region extends from the 5' end of the KICR to beyond its 3' end. It is postulated that the expression of the κ chain is influenced by an enhancer which is located .7 kb 5' of the constant region gene in an area that corresponds to a DNAase-I hypersensitivity site. This region was previously pointed out by members of this laboratory have high sequence conservation between mouse, rabbit and man.

The human J chain gene was cloned in this laboratory and its expression appears to be regulated coordinately with that of immunoglobulin genes in some systems. No sequence homology has been detected between J chain and immunoglobulin genes (Max). Potential regulatory sequences of the J gene have been studied by two approaches. First, an extensive series of gene constructs have been made to assess enhancer and promoter activities of segments from this gene using transient transfection into B cells. Secondly, B cell extracts have been found to bind to segments of DNA 5' region of the gene and gel retardation assays. The characteristics of this binding are currently being investigated.

CHARACTERIZATION OF CELL SURFACE MOLECULES IMPORTANT FOR IMMUNE FUNCTION

Additional studies are carried on in collaboration with other groups to characterize membrane bound molecules especially those present on cytotoxic T lymphocytes that are important in the immune response (Coligan and Koning). In addition, molecules encoded by infectious agents that interact with and are recognized by the immune system are being studied at the structural protein level. These systems currently involve studies of the gene and protein structure of the human T cell receptor/T3 molecular complex. Additional studies involve characterization of the molecules of the AIDS virus which appear to be important to antigenicity of this pathogen and characterization of antigenic variation in Herpes simplex virus type 1 glycoprotein.

Annual Report
Laboratory of Immunogenetics
National Institute of Allergy and Infectious Diseases
October 1, 1985 to September 30, 1986

ADMINISTRATIVE REPORT

During the period covered by this report there has been a turnover of several positions at the postdoctoral level and the group of Dr. Edward Max was moved to the Immunobiology Section of the laboratory from the Immunogenetics Research section. In the Membrane Antigen Section of LIG, Dr. Elliot Cowan left to take a position in the Neurology Institute; Dr. Andrew Lew will leave in August to return to Australia where he has a position at the Walter Eliza Hall Institute. Dr. Erik Lillehoj will leave to accept a position in the Bethesda area. The departures from this section have been replaced by Dr. Diane Handy from the University of Indiana at Indianapolis, by Dr. Frits Koning from The Netherlands and by Dr. Thomas McConnell from the University of Florida. Dr. Nazma Jahan left the laboratory of Dr. Ed Max to accept a position at Georgetown University, Dr. James Flanagan from the University of Minnesota hospitals will join Dr. Max in July. Dr. Roberto Biassoni will join the group of Dr. Eric Long in July. In the Immunogenetics Research section Dr. Patrice Marche left last November to assume a faculty position at the University of Paris and will be replaced this fall by Dr. Ira Lubin from the University of South Carolina at Columbia.

Annual Report
Laboratory of Immunogenetics
National Institute of Allergy and Infectious Diseases
October 1, 1985 to September 30, 1986

HONORS AND AWARDS

Dr. Kindt has presented seminars of his laboratory's work at the University of North Carolina, the University of Alabama, at Duke University, at Georgetown University, at the University of Texas at Dallas, at the Institut Pasteur in Paris, at the University of Paris, at the Tumor Institute in Genoa and at the University of Rome in the past year. He has served as Chairman of a workshop at the International Congress of Immunology and was invited to give a workshop presentation at this meeting. Dr. Kindt continues to serve as Editor for the Journal of Experimental Medicine, for the Journal of Biological Chemistry, and a section editor for immunochemistry and molecular genetics for the Journal of Immunology. He served on review groups for the American Cancer Society, for the Multiple Sclerosis Society, and has served as an ad hoc member of a program projects review group for the National Cancer Institute. In addition, Dr. Kindt has been invited to serve on the scientific review board of Southern Biotechnology Incorporated and has been invited to serve on the board of Scientific Councilors for Oncor, Inc. Dr. Mary Ann Robinson has served as a session chairman at the American Society for Histocompatibility Immunogenetics meeting in fall 1985 and was an invited speaker in a symposium on genetics of disease susceptibility at the 1986 meeting. She has presented at invited seminars at Emory University and was elected to the American Association of Immunologists. Dr. John Coligan has recently been elected a member of the American Association of Biological Chemists. He has presented laboratory data in seminars at Georgetown University, Johns Hopkins University, Harvard University, the University of Leiden and the Netherlands Cancer Institute. Presentations of laboratory data have been made at the International Congress of Immunology and Fifth Cloning Workshop. Dr. Coligan continues to serve as immunochemistry section editor for the Survey of immunological Research and is associate editor for the Journal of Immunology. Dr. John Sogn has presented seminars at Catholic University, at Rutgers University, and at the Naval Medical Research Institute. Dr. Sogn is an associate editor of the Journal of Immunology and will present data at the International Congress of Immunology. Dr. Edward Max is an associate editor of the Journal of Immunology and will present laboratory data at the International Congress of Immunology. Dr. Eric Long has presented invited seminars at Mount Sinai Medical Center and at the Hospital for Joint Diseases in New York. He has been an invited participant in the UCLA Symposium - Molecular Approaches to Developmental Biology and was an invited speaker for the FASEB meeting in St. Louis. In addition, he has been an invited speaker for the MHC Cloning Workshop this year. Members of his laboratory have presented data at the ASHI meeting and at the Gordon Conferences.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00166-09 LIG
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders) Characterization of Rabbit MHC Antigens		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: Thomas J. Kindt OTHER: Marie Christine Rebiere Margherita Bagnato	Chief Visiting Fellow Visiting Fellow	LIG/NIAID LIG/NIAID LIG/NIAID
COOPERATING UNITS (if any) Christian LeGuern, Institut Pasteur, Paris; Annie LeGuern, Institut Pasteur, Paris		
LAB/BRANCH Laboratory of Immunogenetics		
SECTION Immunogenetics Research Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS 3.9	PROFESSIONAL 2.5	OTHER 1.4
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard un-reduced type. Do not exceed the space provided) <p> Current investigations concern the class I and class II genes in the major histocompatibility complex of the rabbit. The class I gene family contains approximately 10 class I genes falling into four subfamilies. Structural data are available for four rabbit cDNA clones, and the genomic clones corresponding to three of these have been completely or partially characterized. Using probes derived from these clones, it was found that the clone pR27 corresponds to a single copy rabbit class I gene that is expressed in the T cell line RL-5 and also in normal rabbit thymus, but not in the other tissues studied. The pR27 transcript is larger than normal class I transcripts apparently because certain introns have not been spliced out. Studies are under way using S1 nuclease mapping to determine the precise splicing patterns in the transformed cell line (RL-5) as well as in normal thymus. Studies of rabbit class II genes have included complete sequence analysis of a cDNA and a genomic clone corresponding to the rabbit DQ alpha homologue. This gene is highly homologous to the HLA-DQ of man and is quite similar to the H-2 I-A alpha gene of the mouse. The RLA-DP2 alpha gene has been partially sequenced and data indicate that it may be a homologue of the human gene designated HLA-DZ. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00168-09 LIG
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders) Cell Surface Markers of Rabbit Lymphocytes		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: Thomas J. Kindt	Chief	LIG/NIAID
OTHER: Patrice Marche	Visiting Fellow	LIG/NIAID
COOPERATING UNITS (if any) P.A. Cazenave, Institute Pasteur; David Sachs, NCI.		
LAB BRANCH Laboratory of Immunogenetics		
SECTION Immunogenetics Research Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS 2.0	PROFESSIONAL 0.6	OTHER 1.4
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard, unreduced type. Do not exceed the space provided.) <p> The rabbit T cell line, RL-5, which was derived from the B/J inbred strain of rabbits by transformation with the virus <u>Herpes ateles</u>, was used for initial studies concerning the T cell receptor genes of the rabbit. Probes corresponding to the constant region of the murine T cell receptor alpha and beta chain genes were used to screen genomic and cDNA libraries constructed from the RL-5 line. The cDNA library yielded two full-length alpha transcripts that were isolated and their sequences determined. It is unexpected to find two transcripts for a T cell receptor gene in a single clonal cell line. One of the transcripts contained a single base pair deletion in the variable region which placed the remainder of the transcript out-of-phase. The other transcript encoded a complete alpha chain corresponding to a leader sequence, variable region, J region, constant and 3' untranslated regions. Variable region probes from these two transcripts were used to probe genomic library and each was found to correspond to multigenic families. Studies on the beta chain transcripts produced by the RL-5 cell line indicated the presence of two transcripts, one 1.3 kb in length, the other approximately 1.0 kb in length. These were sequenced, the larger was found to correspond to a complete T cell beta chain. The other is an incomplete transcript of a beta chain and lacks a variable region. Its sequence is presently under detailed analysis. A probe derived from the variable region of the beta chain cDNA clone detected variable region genes in DNA samples from man, rabbit and pig, but no bands were detected when samples from mouse, rat and hamster were probed under similar conditions. Comparisons of constant region genes from man, mouse and rabbit indicate a high degree of conservation within species and significant, but considerably lower conservation among the species studied. The 3' untranslated regions of the beta genes however, showed conservation among the C beta 1 and the C beta 2 regions from the different species. No such identification of beta 1 or beta 2 sequence was possible in comparisons of the constant coding region. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00169-09 LIG																				
PERIOD COVERED October 1, 1985 to September 30, 1986																						
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Analysis of Murine and Human Transplantation Antigens and Genes																						
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 40%;">John E. Coligan</td> <td style="width: 40%;">Senior Investigator</td> <td style="width: 10%;">LIG/NIAID</td> </tr> <tr> <td>OTHER:</td> <td>Elliot Cowan</td> <td>Staff Fellow</td> <td>LIG/NIAID</td> </tr> <tr> <td></td> <td>Andrew Lew</td> <td>Visiting Fellow</td> <td>LIG/NIAID</td> </tr> <tr> <td></td> <td>Erik Lillehoj</td> <td>Staff Fellow</td> <td>LIG/NIAID</td> </tr> <tr> <td></td> <td>Diane Handy</td> <td>Guest Worker, Staff Fellow</td> <td>LIG/NIAID</td> </tr> </table>			PI:	John E. Coligan	Senior Investigator	LIG/NIAID	OTHER:	Elliot Cowan	Staff Fellow	LIG/NIAID		Andrew Lew	Visiting Fellow	LIG/NIAID		Erik Lillehoj	Staff Fellow	LIG/NIAID		Diane Handy	Guest Worker, Staff Fellow	LIG/NIAID
PI:	John E. Coligan	Senior Investigator	LIG/NIAID																			
OTHER:	Elliot Cowan	Staff Fellow	LIG/NIAID																			
	Andrew Lew	Visiting Fellow	LIG/NIAID																			
	Erik Lillehoj	Staff Fellow	LIG/NIAID																			
	Diane Handy	Guest Worker, Staff Fellow	LIG/NIAID																			
COOPERATING UNITS (if any) W. Biddison, NINCDS, NIH; T. Hansen, Washington University, St. Louis; E. Wakeland, University of Florida; D. Margulies, NIAID																						
LAB/BRANCH Laboratory of Immunogenetics																						
SECTION Membrane Antigen Structure Section																						
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205																						
TOTAL MAN-YEARS 4.1	PROFESSIONAL: 2.85	OTHER 1.25																				
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews																						
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Human and murine major histocompatibility complex encoded class I and II molecules that are integrally involved in the presentation of antigen to cytotoxic and helper T lymphocytes, respectively, are isolated and their molecular properties are studied. The goal of these studies is to gain a molecular understanding of their functional and antigenic properties. Analysis of the HLA-A3 variant (E1) that is altered in its ability to present influenza antigens to CTL has revealed that it was most likely derived by a gene conversion event. Such events appear to be the predominant mechanism for generating diversity among these genes. In the mouse studies, it has been determined that class I molecules achieve additional diversity through the use of alternative transcripts of the same gene. The alternative forms of certain class I molecules appear to be differentially expressed in T and B cells suggesting that they may have functional significance. Factors controlling expression of the murine soluble class I molecule (Q10) were investigated. Serum levels, under control of the H-2D region, varied significantly for different haplotypes and were affected by various external factors. Additional studies in the mouse have revealed a second family of D-region molecules representing several distinct haplotypes. This so called Dw3-family was found to be quite distinct from the previously defined Ld-family of molecules. Class II molecules through their interaction with antigens are important for regulation of the antibody response by T helper cells. Structural variations in IAk molecules involved in this regulatory process have been localized to the alpha 1 and beta 1 domains.</p>																						

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00170-09 LIG
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Studies of Human Histocompatibility Antigens		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Eric O. Long	Visiting Scientist LIG/NIAID
OTHER:	Rafick Sekaly	Visiting Fellow LIG/NIAID
	Sandra Rosen-Bronson	Staff Fellow LIG/NIAID
	Roberto Blassoni	Visiting Fellow LIG/NIAID
COOPERATING UNITS (if any)		
Hugh McDevitt, Stanford University, Philippa Marrack, National Jewish Hospital, Denver		
LAB BRANCH		
Laboratory of Immunogenetics		
SECTION		
Immunogenetics Research Section		
INSTITUTE AND LOCATION		
NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS	PROFESSIONAL	OTHER
4.5	3.25	1.25
CHECK APPROPRIATE BOXES		
<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unspaced type. Do not exceed the space provided.)		
<p> The human immune response genes are located in the D region of the major histocompatibility complex (MHC). They encode the alpha and beta chains of the HLA-DR, -DQ and -DP class II antigens. The aim of this project is to define by a molecular approach how many functional class II genes exist and to express individual class II antigens in cell lines by DNA-mediated gene transfer in order to analyze the interaction between class II antigens and T lymphocytes. cDNA clones in eukaryotic expression vectors have been obtained for the alpha and beta chains of the DP, DQ and DR antigens. Various murine, simian and human cell lines have been transfected with these clones and express defined human class II antigens at their surface. This expression system has allowed us to show that the invariant chain, a glycoprotein associated intracellularly with class II antigens, was not necessary for cell surface expression of class II antigens. It was also possible to show that the DR antigens interact with the T4 molecules, a cell surface antigen expressed by a subset of T lymphocytes (collaboration with P. Marrack). Transfected cell lines expressing class II antigens are able to present foreign viral antigens to T lymphocytes with appropriate specificity for viral antigens and MHC molecules. This system can be used to define the elements important for antigen-recognition by T lymphocytes. New class II genes have been identified and isolated as complete cDNA clones; they are called DQ beta and DZ alpha. Whereas DZ alpha is expressed like a typical class II gene in B cells, activated T cells and cells treated with gamma interferon, DQ beta expression is restricted to B cells. By use of pulsed-field gel electrophoresis it was possible to determine the complete linkage of class II genes at the molecular level and to precisely map the DQ beta and DZ alpha genes within the 1100 kilobases present in the D region of HLA (collaboration with H. McDevitt). The availability of complete cDNA clones for DQ beta and DZ alpha makes it possible to analyze these putative new class II antigens at the protein level. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00171-09 LIG
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Genetic Studies on Rabbit Immunoglobulins and Other Serum Proteins		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: John A. Sogn Senior Investigator LIG/NIAID OTHER: Fathia Mami Visiting Fellow LIG/NIAID		
COOPERATING UNITS (if any) J. Coe, RML, NIAID; A.M. Lewis, LIP, NIAID; P. Tucker, University Texas, Southwestern Medical School, Dallas, TX		
LAB/BRANCH Laboratory of Immunogenetics		
SECTION Immunogenetics Research Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 2.2	PROFESSIONAL: 1.5	OTHER: 0.7
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The class I major histocompatibility antigens in the hamster are unique among species that have been closely examined because they appear to be nonpolymorphic. The organization of the class I gene complex is now under investigation to attempt to determine the reason for this unusual behavior. A number of class I genes have been cloned from a genomic library and mapped. The identity of the expressed cell-surface antigens will be determined by examining cDNA clones. The functional significance of changes in class I expression is being investigated in hamster and mouse cells transformed with oncogenic and nononcogenic adenoviruses. The oncogenic adenovirus 12 appears to rely on elimination of class I antigen expression in the transformed cell to escape host detection. The nononcogenic adenoviruses 2 and 5 have no such effect. Ad2 and Ad5 transformants expressing low levels of class I antigens have been found but they are still nontumorigenic. In addition, adult adaptation of an Ad2 transformant led to high tumorigenicity without a change in class I expression. Thus, class I antigen expression is irrelevant to tumorigenicity by the nononcogenic adenoviruses. Hamster female protein has been examined with respect to its disulfide bond arrangement. Each subunit has two cysteine involved in an intramolecular disulfide bond. The third cysteine in each subunit is the most carboxy-terminal one and is present in the reduced state. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00173-09 LIG
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Control of Gene Expression		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Edward E. Max	Commissioned Officer LIG/NIAID
OTHER:	Jeffrey Gimble	Medical Staff Fellow LIG/NIAID
	Nazma Jahan	Visiting Fellow LIG/NIAID
	James Flanagan	Medical Staff Fellow LIG/NIAID
	Mary Ann Robinson	Senior Staff Fellow LIG/NIAID
COOPERATING UNITS (if any) O. Wesley McBride, Laboratory of Biochemistry, NCI; Cynthia Morton, Department of Genetics, Harvard Medical School; Nicoletta Sacchi, Laboratory of Molecular Oncology, NCI		
LAB/BRANCH Laboratory of Immunogenetics		
SECTION Immunogenetics Research Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS 4.1	PROFESSIONAL 2.8	OTHER 1.3
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unindented type. Do not exceed the space provided.) <p> Mechanisms of gene expression control are being investigated for two genes that are active in human B lymphocytes: the immunoglobulin kappa light chain and J chain genes. The expression of the kappa gene is known to be influenced by an enhancer located about 0.7 kb 5' of the constant region gene; this corresponds to the position of a B cell-specific DNAase I hypersensitivity site and a segment of about 0.13 kb showing high sequence conservation between mouse, rabbit and man--the Kappa Intron Conserved Region (KICR). We have employed the high-resolution electroblotting technique originally developed for genomic sequence analysis to examine the accessibility of this region to nucleases and to dimethylsulfate (in vivo footprint analysis). The experiments reveal a ~0.25 kb segment of DNAase I hypersensitivity and accessibility to restriction endonucleases that extends from the 5' end of the KICR to beyond its 3' end. Two sites of enhanced dimethylsulfate reactivity are located within dyad symmetries at the 5' end of the KICR, consistent with the binding of regulatory proteins to these positions in vivo. We are currently using an exonuclease protection method to detect in vitro the binding of nuclear extract proteins to the same positions and hope eventually to purify such proteins. </p> <p> The human J chain gene was cloned in our laboratory; its expression appears to be regulated coordinately with that of immunoglobulin genes in some systems although no sequence homology has been detected between J chain and immunoglobulin genes. Potential regulatory regions of the gene have been studied by two approaches. First, an extensive series of gene constructs have been made to assess enhancer and promoter activities of segments from the gene using transient transfection into B cells. Second, B cell extracts have been found to bind to segments of DNA 5' of the gene in gel retardation assays; the characteristics of this binding are currently being investigated. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00180-08 LIG
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Properties of Transformed Rabbit Cell Lines		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: John A. Sogn Senior Investigator LIG/NIAID OTHER: Henrietta Kulaga Staff Fellow LIG/NIAID		
COOPERATING UNITS (if any) G Marti, Hematology Branch, Clinical Center, NIH		
LAB/BRANCH Laboratory of Immunogenetics		
SECTION Immunogenetics Research Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 2.7	PROFESSIONAL: 1.5	OTHER: 1.2
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> This project involves the pattern of expression of rabbit class II major histocompatibility complex antigens. Previous work from this laboratory demonstrated the existence of five genes encoding class II alpha chains in the rabbit. These have now been surveyed for mRNA expression in normal and pathological tissues of the rabbit. The results obtained are for the most part consistent with the tentative assignments of these genes as homologues of human alpha chain genes by strength of hybridization. The RLA genes referred to as RLA-DR alpha, RLA-DQ alpha and RLA-DP1 alpha are expressed with a pattern identical to that observed in the human for the HLA genes of the same name. Expression studies of RLA-DP2 alpha demonstrate a larger (3.6 kb) transcript, suggesting homology to the poorly understood human gene HLA-DZ alpha. This has now been borne out by sequence analysis. Northern blot analysis of RLA-DN alpha shows nothing resembling a mature class I alpha chain transcript but instead a very large transcript expressed most strongly in heart. This phenomenon is under study. A panel of class II-positive rabbit continuous cell lines has been established largely but not exclusively by <u>in vitro</u> viral transformation. Most of the viral transformants are macrophage-like in properties, but very recent experiments with human T lymphotropic virus I (HTLV-I) suggest that rabbit cells can be readily transformed by this virus. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00352-04 LIG
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Characterization of Cell Surface Molecules Important for Immune Function		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: John E. Coligan OTHER: Frits Koning Andrew Lew	Senior Investigator Guest Worker Visiting Fellow	LIG/NIAID LIG/NIAID LIG/NIAID
COOPERATING UNITS (if any) Cox Terhorst, Dana-Farber Cancer Institute; Max Essex, Harvard School of Public Health; R. Nairn, University of Michigan.		
LAB/BRANCH Laboratory of Immunogenetics		
SECTION Membrane Antigen Structure Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205		
TOTAL MAN-YEARS 3.2	PROFESSIONAL: 1.9	OTHER 1.25
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.) <p>Multiple molecules exist on the surface of lymphocytes which are important for the development of the immune response. A major goal of these studies is to identify and structurally characterize these membrane-bound molecules, especially those present on cytotoxic T lymphocytes. Concordantly, it is important to have an understanding of the molecules encoded by infectious agents which are recognized by the immune system. Thus, the nature of the antigens in several viruses posing serious health problems is being investigated. Project areas include: (1) studies on the gene and protein structures of the human T cell receptor - T3 molecular complex, (2) characterization of the molecules of antigenic importance expressed by the AIDS virus, and (3) studies on antigenic variation in <u>Herpes simplex virus</u> type 1 (HSV-1) glycoproteins.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00389-03 LIG
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Molecular Genotype Analyses in HLA Recombinant Human Families		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Mary Ann Robinson Senior Staff Fellow LIG/NIAID OTHER: Thomas J. Kindt Chief LIG/NIAID		
COOPERATING UNITS (if any) D. Bernard Amos, Duke University; Clifton Lane, LIR, NIAID		
LAB/BRANCH Laboratory of Immunogenetics		
SECTION Immunogenetics Research Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 1.85	PROFESSIONAL: 1.05	OTHER: 0.8
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> In order to investigate the genetic control of human immune responses, the inheritance of several genes known to play important roles in a variety of immune processes has been analyzed in human families. Probes corresponding to HLA class I, class II (DR alpha, DR beta, DQ alpha, DQ beta, DO alpha and DP beta) and T cell antigen receptor (TcR) alpha chain (C + 3' untranslated region and V4) and beta chain (C and V8.1) genes were used in Southern blotting analyses of DNA samples from the members of eight families. Polymorphic restriction fragments hybridizing with a TcR beta constant (C) region probe were observed to segregate in six of the eight families and certain haplotype assignments could be made. Two additional polymorphisms were observed with a probe corresponding to a variable (V) gene segment and were used to complete assignment of haplotypes for nearly all individuals. Different combinations of the C and two V region markers can result in eight possible distinct haplotypes and all but one of these combinations was observed in the parents of the families studied. While the data regarding segregation of polymorphic V and C fragments provided support for linkage of V and C region gene segments, the diversity of haplotypes at the population level suggests that recombination has occurred between V and C region gene segments and among members of the V region subfamily marked by the probe used. Similar results were obtained using TcR alpha V and C+3' untranslated probes. Human TcR alpha chain haplotypes appears to be quite polymorphic. These polymorphisms will provide useful markers that will facilitate linkage studies mapping studies, genetic analyses of T cell function, and the means of evaluating the role of TcR genes in disease susceptibility. </p>		





LABORATORY OF IMMUNOLOGY
1986 Annual Report
Table of Contents

<u>Z01-AI</u> <u>Project Number</u>		<u>Page</u>
Summary		5- 1
00030-18	Antigen Recognition and Activation of Immunocompetent Cells - Paul	5-14
00035-11	Specificity in Immune Responses - Inman	5-15
00036-21	Ig Genetics: Ontogeny and Differentiation of Cells of the Rabbit Immune System - Mage	5-16
00147-11	The Mechanism of Activation of Thymus-Derived Lymphocytes - Schwartz	5-17
00148-11	Immunological Studies of Guinea Pig L ₂ C Leukemia - Green	5-18
00186-13	Pathogenesis of Autoimmunity in Inbred Strains in Mice - Chused	5-19
00203-07	Applications of Flow Cytometry - Chused	5-20
00223-05	Cellular Interactions in the Immune Response - Schwartz	5-21
00224-05	Monoclonal Antibodies as Probes for T Cell Activation - Shevach	5-22
00226-05	Rabbit Allotypes: Structure, Organization and Regulated Expression of Ig Genes - Mage	5-23
00229-04	Studies on Lymphocyte Differentiation - Cohen	5-24
00259-04	Ia Molecules and Immune Response Genes - Paul	5-25
00349-04	Structure and Function of Murine Class II MHC Genes and Gene Products - Germain	5-26
00394-03	Molecular Genetic Analysis of Lymphocyte Function - Margulies	5-27
00400-02	Immunoregulatory Disorders in Systemic Lupus Erythematosus - Green	5-28

00403-03	Molecular Genetic Analysis of T Cell Receptor Structure and Repertoire - Germain	5-29
00425-02	Lymphocyte Physiology - Chused	5-30
00426-01	Organization of the Antigens in the Plasma Membranes of the Lymphocyte Clones - Sitkovsky	5-31
00427-02	Antigen-Specific and Antigen-Nonspecific Cellular Cytotoxicity - Sitkovsky	5-32

PHS-NIH
Summary Statement
Office of the Chief
Laboratory of Immunology
October 1, 1985 through September 30, 1986

Introduction

The Laboratory of Immunology is concerned with the elucidation of the fundamental mechanisms underlying immunologic responses. It had made rapid progress through the use of three new technologies which are creating a revolution in immunologic science. These are the use of monoclonal antibodies, the adaptation of techniques of molecular genetics to immunologic problems, and the use of long-term lines of cloned normal and transformed lymphocytes. The continued use and major improvement of these approaches should allow solution of many of the major problems which have concerned immunologists and should provide important advances in the efforts to more precisely regulate the normal and the disordered immune response.

Sequences of Rabbit T Cell Receptor Genes Suggest Strong Evolutionary Conservation of Variable Regions

Studies in the Laboratory of Immunology by Davis et. al. and in other laboratories have identified the genes encoding T cell receptor polypeptide chains. This has made possible detailed examination of the structure of the genes and proteins involved in antigen recognition by T cells. Studies of the structure of the T cell receptor genes in other species should allow an examination of the evolution of this important gene family. Laboratory of Immunology scientists have identified genomic clones for the genes encoding a set of rabbit T cell receptor β chains, have examined their genomic organization and have obtained nucleotide sequences of these genes. Of the three rabbit V_{β} genes examined, each demonstrates strong sequence homology with mouse and/or human V_{β} genes but one of them differs remarkably from the other two. This suggests that the V_{β} genes duplicated prior to rabbit-mouse radiation from a common ancestor and that there is evolutionary conservation of portions of the protein sequence of certain members of the V_{β} family. This evolutionary conservation is interesting in light of evidence in the rabbit, as in the human and the mouse, that little or no somatic mutation of T cell receptor V_{β} genes occurs, suggesting that the structure of T cell receptors may be selected for binding specificity rather than simply for maximum repertoire diversity (Lamoyi and Mage, LI/NIAID).

Molecular Events in Thymocyte Differentiation

Cells of the T lineage undergo a complex series of differentiation and selection events within the thymus through which an immature precursor population is converted into a set of

functionally active, clonally diversified cells with a broad repertoire for the corecognition of antigen and MHC molecules. Recent studies of the cell surface markers of developing thymocytes have established some of the properties of the progenitor cells in the thymus. During the past year, Laboratory of Immunology scientists, working with other NIH colleagues, have examined the pattern of gene expression in thymocytes of developing mouse embryos. At 12 days of fetal life, the thymus contains only 1000 to 2000 cells of which 60% express mRNA for Thy1, detected by in situ hybridization. Such Thy1-positive cells appear to be the most primitive thymic lymphocytes. At that time, mRNA for the T cell receptor γ -chain is expressed in a similar percentage of cells, but no cells expressed mRNA for either the β or α chains of the T cell receptor. β chain mRNA first appears at 12.5 days of gestation and the frequency of cells expressing this mRNA then rises rapidly. Cells do not express T cell receptor α chain mRNA until day 16 of fetal life, which is the time at which γ chain mRNA expression diminishes.

A striking proliferation of thymocytes occurs between days 13 and 15, which correlates with the time of onset of expression of mRNA for interleukin-2 (IL-2) and for the IL-2 receptor; this suggests that intrathymic IL-2 production, possibly autocrine, may be a powerful stimulant of thymocyte proliferation.

These studies have indicated that immature T cell progenitors fail to express either the Lyt2 or L3T4 markers and that at 14-15 days of development these progenitors become sensitive to exogenous stimuli which cause them to develop into Lyt2⁺, L3T4⁺ T cells. By contrast, the expression of both markers may very well represent a distinct T cell differentiation pathway, possibly leading to cell death. The analysis of gene expression in thymocyte subpopulations of timed developmental state promises to provide a detailed picture of T lymphocyte differentiation and diversification (Pardoll, Hua, Fox, McCoy, Germain, and Schwartz, LI/NIAID; Fowlkes and Asofsky, LMI/NIAID; Kruisbeck, BRMP/NCI).

Thy1⁺ Dendritic Epidermal Cells Contain mRNAs for T Cell Receptor Chains, for the IL-2 Receptor and for Thy1

Dendritic epidermal cells consist of two populations, one of which expresses the Thy1 antigen and lacks class II MHC molecules. In many respects, these cells resemble immature thymocytes, raising the possibility that the epidermis may function as a site of extrathymic T cell differentiation. Laboratory of Immunology scientists have now established cell lines from this population, which express the cell surface phenotype of the resident Thy1⁺ dendritic epidermal cells. These cells express mRNA for the α , β , and γ chains of the T cell receptor, for the IL-2 receptor, and for Thy1. In two of the lines, the majority of α and β mRNAs were smaller than those

which code for complete chains, consistent with truncated, incompletely rearranged forms. These results are similar to those observed for developing thymocytes and strengthen the possibility that an extrathymic site of T cell maturation exists in the skin (Stingl and Shevach, LI/NIAID).

Expression and Function of Transfected T Cell Receptor Genes

The T cell receptor has been shown to contain α and β chains which both possess variable regions and are believed to form the antigen-combining site of the receptor. The $\alpha\beta$ heterodimer is associated with a complex of membrane associated polypeptides, designated the T_3 complex, which is believed to play a critical role in signal transduction. In order to examine the requirements for surface expression of the receptor complex and to test predictions about the binding activity of the receptor, Laboratory of Immunology scientists have utilized a DNA-mediated gene transfer approach, introducing mouse genes into mutant or normal human T cell lines. Using this approach, they established that an intact $\alpha\beta$ heterodimer is required for surface expression of the T_3 complex. This was achieved by introducing a mouse β chain gene into a human cell line which failed to transcribe its own β chain gene and which did not express either the $\alpha\beta$ heterodimer or the T_3 complex. The resultant transfected cell expressed on its surface a mouse β -human α heterodimer and the T_3 complex. Furthermore, other transfectants were obtained in which human α -mouse β heterodimers and mouse α -mouse β heterodimers were expressed with the human T_3 complex. In each case, the cells could be triggered to secrete IL-2 with anti- T_3 antibody plus phorbol ester and, in transfectants expressing the mouse 2B4 α chain, a monoclonal anti-2B4 clonotypic antibody plus phorbol ester induced IL-2 production. These experiments establish that human and mouse T cell receptor and T_3 polypeptides can successfully interact and create a complex capable of transmitting a transmembrane signal. Finally, transfected human cells expressing mouse α and β chains obtained from a cytochrome c-I-E^K-specific T cell hybridoma acquired the ability to respond to cytochrome c presented by I-E^K-bearing mouse antigen-presenting cells. These results indicate that the $\alpha\beta$ heterodimer completely defines the specificity of the MHC-restricted antigen specific T cell (Saito, Avigan, and Germain, LI/NIAID).

T Cells are Tolerized By Exposure to Antigen and Class II MHC Molecules without Accessory Cell Function

T cell clones cospecific for a cytochrome c peptide (residues 81-104) and E^K class II molecules produce interleukin-2 (IL-2) and proliferate when exposed to the peptide and intact antigen-presenting cells of the I-E^K type. By contrast, neither IL-2 production or entry into S phase is noted when T cell clones are stimulated with cytochrome c peptide plus I-E^K in planar lipid membranes or on spleen cells treated with the chemical crosslinker 1-ethyl-3-(3-dimethyl

aminopropyl) carbodiimide (ECDI). Laboratory of Immunology scientists have now shown that this unresponsiveness correlates with the induction of a state of immunologic tolerance in that T cell clone. These cells fail to respond to subsequent stimulation with cytochrome c peptide presented by intact I-E^K-bearing antigen-presenting cells. The induced failure to respond lasts for at least 8 days and does not represent a total inactivation of the cells since they can respond to added IL-2.

Induction of the tolerant state requires the presence of both the cytochrome c peptide and the I-E^K molecule, indicating that the specificity of tolerance induction and of activation is similar. T cells exposed to a tolerogenic signal show normal induction of mRNA for the β chain of the T cell receptor, partial induction of IL-3 and of the IL-2 receptor, and essentially no induction of IL-2. As noted above, addition of IL-2 allows these cells to enter S phase, suggesting that the failure to produce IL-2 is critical to the induction of the tolerant state. These experiments establish a powerful system in which the biochemical basis of induction of antigen-specific, MHC-restricted tolerance can be precisely examined (M. Jenkins, H. Quill, K. Ogasawara, and R. H. Schwartz, LI/NIAID).

Elevation of Lymphocyte $[Ca^{2+}]_i$ As a Result of Receptor-Mediated Signalling

The recent development of calcium-binding dyes which can be used to measure intracellular free calcium concentration $[Ca^{2+}]_i$ has made possible the examination of the role of calcium in a variety of cell activation systems. The application of this methodology to flow cytometry provides a particularly powerful approach to the analysis of the dynamics of lymphocyte activation. Laboratory of Immunology scientists have examined $[Ca^{2+}]_i$ elevation in both B and T lymphocytes in response to receptor cross-linkage, using flow cytometry of cells loaded with Indo-1. Normal B cells and B lymphoma cells to which anti-immunoglobulin (Ig) has been added, to cross-link membrane Ig, show an increase in $[Ca^{2+}]_i$ from 100nM, in the resting state, to 2-3 μ M within 10 seconds; in 2 minutes, this falls to 300-700nM and then gradually declines over several hours. Peak responses can be obtained with concentrations of anti-Ig sufficient to cross-link only 3% of the mIg. Anti-IgD antibodies stimulate a more striking increase in $[Ca^{2+}]_i$ than do anti-IgM antibodies and the elevation in $[Ca^{2+}]_i$ is prolonged if F(ab')₂ anti-Ig is used or if the Fc γ receptor is blocked with the 2.4.G2 monoclonal anti-Fc γ receptor antibody. These results suggest that membrane IgD may be superior to membrane IgM in signal transduction although other explanations such as affinity of the antibodies and surface density need to be excluded. In addition, these results indicate that cross-linkage of the Fc γ receptor to membrane Ig down regulates the activation signal, probably by diminishing the activity of phosphatidyl inositol bis-4,5-diphosphate diesterase. T cells show a similar increase in $[Ca^{2+}]_i$ in response to anti-receptor antibody or to processed antigen on presenting cells

which express class II MHC molecules. In the latter case, all responding T cells are found to have formed conjugates with antigen-presenting cells and prolonged elevation of $[Ca^{2+}]_i$ requires that the T cell remain in such a conjugate. These results indicate that cross-linkage of membrane antigen-binding receptors of both B and T lymphocytes profoundly alters $[Ca^{2+}]_i$, and strongly suggest that such alterations are critical to lymphocyte activation in physiologic conditions (Chused and Ishida, LI/NIAID; Ji, LMI/NIAID; Samelson and Klausner, CBMB/NICHD; Tsien, University of California, Berkeley; Finkelman, USUHS).

The Amino Terminal Portion of the Class II MHC β Chain Controls Selective Chain Pairing

Class II MHC molecules are co-recognized with antigenic peptides by T cell receptors. Recent evidence indicates that these molecules bind to antigenic peptides and that this binding creates the complex recognized by the T cell. This critical function of class II molecules has made important a structural analysis of class II molecules. Laboratory of Immunology scientists have used transfection of L cells with genes for class II α and β chains to study the rules which govern the pairing of the two constituent chains of the class II molecules. These experiments have resulted in several previously unrecognized and potentially very important insights. Among these are the following: in every instance genes encoded on the same chromosome give more efficient membrane expression of class II molecules than do genes obtained from allelic chromosomes. The β chain region which controls this selective pairing of α and β chains is found in the N-terminal fifty amino acids of the molecules. Haplotype mismatched pairs give variable levels of expression, ranging from undetectable ($A_B:A_K$) to virtually the same as cis pairs ($A_K:A_B$). Indeed, the very same α region of the β chain also regulates pairing of α and β chains encoded by genes at different loci (i.e. the I-A and I-E loci, respectively). These results have lead to the conclusion that the A_B and A_K domains physically interact and that a process of coevolution of cis-encoded A_K and A_B has occurred such that high efficiency of surface expression of class II molecules dependent on domain interaction is preserved even as allelic polymorphism accumulates. The lack of recombination between A_B and A_K genes probably reflects a strong selective pressure to preserve highly efficient coexpression of surface class II MHC molecules (Braunstein, Sant, Ronchese, Lechler and Germain, LI/NIAID).

Production of Soluble Recombinant Class I MHC Molecules and Use in Structural and Functional Studies

Class I major histocompatibility complex (MHC) molecules function as co-recognition elements for cytotoxic T cells and are directly recognized by allogeneic T cells. These structures play important

roles in the allograft rejection process. Analysis of their structure and function has been hampered by the fact that they are integral membrane proteins which have limited solubility in aqueous conditions and can not be easily prepared in large amounts. Laboratory of Immunology scientists have now developed a strategy for the production of large quantities of soluble class I MHC molecules. It is based on the existence of a non-polymorphic murine class I molecule, Q10, which is coded for by a gene that has a 13 nucleotide deletion in its transmembrane exon and which, accordingly, is a secreted rather than membrane-bound. Chimeric genes have now been constructed possessing the 5' half of H-2D^d and the 3' half of Q10. Upon introduction into L cells, a soluble protein is secreted which contains the two amino terminal domains of the H-2D^d molecule and which can be easily purified by affinity chromatography. This soluble material is being used to establish conditions for crystallization in an effort to determine the three dimensional structure of class I MHC molecules and for direct binding studies to T cell receptor to allow thermodynamic measurements of T cell receptor-ligand interactions. Such measurements have not been possible with previous techniques (Margulies, McCluskey, Ramsey and Lopez, LI/NIAID).

B Cell Stimulatory Factor-1 (BSF-1): Purification and Development of a Receptor-Binding Assay

BSF-1 was initially described by Laboratory of Immunology scientists as a required co-stimulant in the proliferative response of resting B lymphocytes to low concentrations of anti-immunoglobulin (Ig) antibody. Last year a monoclonal antibody was prepared against BSF-1, a technique for its partial purification was developed, and important new insights into its mode of action were obtained emphasizing its capacity to act upon resting B cells. During the period covered by this report, BSF-1 was purified to homogeneity by a simple, two-step technique. Supernatant fluids (Sn) were obtained from cells of the T cell line EL-4 which had been stimulated with phorbol esters. These Sn were adsorbed to and eluted from an anti-BSF-1 affinity column and then subjected to reversed phase high pressure liquid chromatography. Purified BSF-1 with an apparent molecular wt. of 20,000 was obtained. The N-terminal amino acid sequence of this molecule was His, Ile, Gly, ..., Asp, Lys, Asn, His, Leu, Arg, Glu, Ile, Ile, Gly, Ile, Leu, Asn, Gly, Val, Thr, Gly, Glu, Gly. This sequence matches that inferred from the nucleotide sequence of cDNA clones obtained in other laboratories. Furthermore, a rabbit antibody to a synthetic peptide was prepared consisting of amino acids 100-113, inferred from the nucleotide sequence. This antibody immunoprecipitated purified BSF-1 providing very strong evidence that the purified protein and the cDNA clone represented the same molecule.

BSF-1 was iodinated without destroying its biologic activity. ¹²⁵I-BSF-1 bound to spleen cells in a saturable and inhibitable manner. Scatchard analysis indicated that spleen cells had ~500

receptors/cell with an binding constant of $2.9 \times 10^{10} \text{ M}^{-1}$. BSF-1 receptors were found on resting B and T lymphocytes and increased in number, from 2 to 8 fold, upon lymphocyte activation. In addition to B and T cells and to tumors of these cell types, receptors were found on macrophage, mast cell, and myeloid lines, indicating that BSF-1 may be a factor with broad significance for hematopoietic lineage cells. ^{125}I -BSF-1 was cross-linked to its receptor with the bifunctional reagent disuccinimidyl suberate. The complex had an apparent size of $\sim 80,000$, suggesting that the BSF-1 receptor, or one chain of the receptor, weighed $\sim 60,000$ daltons (Ohara and Paul, LI/NIAID; Coligan and Maloy, LI/NIAID).

B Cell Stimulatory Factor-1 Has Actions on A Broad Range of Hematopoietic Lineage Cells

Purified BSF-1 is known to be a potent costimulant of entry of B cells into the S phase of the cell cycle and to act on resting B cells to induce the heightened expression of class II major histocompatibility complex (MHC) molecules. Last year, it was shown that BSF-1 causes B cells stimulated with lipopolysaccharide to secrete IgG1 and that the factor previously designated BCDGy is actually BSF-1. During the past year it was shown that BSF-1 also causes the production of IgE in this culture system. Furthermore, administration of monoclonal anti-BSF-1 antibody to mice at the same time they received an injection of Nippostrongylus brasiliensis larvae markedly inhibits the rise in serum IgE normally caused by such helminths. This strongly suggests that BSF-1 plays a major role, in vivo, in regulation of serum IgE in response to parasitic infestation and, possibly, to allergens.

BSF-1 also has potent actions on normal T cells and T cell lines. Highly purified resting T cells stimulated with BSF-1 and phorbol esters are stimulated to proliferate. This response is density independent, does not depend upon other cell types, and is demonstrated by both L3T4^+ and Lyt2^+ T cells. Activated T cells enter S phase in response to BSF-1 alone. Efforts are underway to determine whether BSF-1 acts as a T cell growth factor, analogous to interleukin-2 (IL-2) or whether its mode of action depends upon the expression of IL-2 receptors and the production of IL-2.

BSF-1 costimulates, with recombinant interleukin-1 (IL-1) or erythropoietin (rEpo), the appearance in soft agar of megakaryocytic colonies from anti-Thy1-treated bone marrow cells. With rEpo, it also causes the appearance of erythroid colonies. These results indicate that BSF-1 has broad action on hematopoietic lineage cells. It has been proposed that its principal activity is as a competence or "co-competence" factor (Brown, Hu-Li, Snapper, Peschel, Green, Ohara, and Paul, LI/NIAID; F. Finkelman, USUHS; R. Coffman and T. Mossmann, DNAX).

Anti-Ly-6 Antibodies Activate T Lymphocytes

The clarification of membrane structures which are involved in lymphocyte growth control has been markedly aided by the development and characterization of monoclonal antibodies specific for cell surface determinants. Ly-6 is a complex alloantigen, determined by genes on chromosome 6. As many as five distinct alloantigens exist for each of the recognized haplotypes. These alloantigens are expressed differentially on various cell types. Laboratory of Immunology scientists have developed a new monoclonal anti-Ly-6 antibody, D7, which triggers proliferation of resting T cells in the presence of phorbol myristate acetate (PMA). D7 induced responses by both Lyt2^+ and L3T4^+ T lymphocytes and it enhanced responses to antigens and alloantigens, but not to mitogens. The mechanism through which Ly-6 causes lymphocyte activation has not yet been clarified, but two major possibilities are now under consideration. Ly-6 may either represent a new signal transmission pathway or it may function through, and possibly be part of, the T3-Ti complex (Shevach, Malek and Ortega, LI/NIAID).

Exocytosis of A Trypsin-Type Serine Esterase from Cytotoxic T Lymphocytes as a Result of Receptor-Mediated Triggering

Destruction of target cells by cytotoxic T lymphocytes (CTL) has been proposed to involve secretion of lytic molecules by the CTL. Laboratory of Immunology scientists, working with colleagues in the National Cancer Institute, have obtained evidence that a trypsin-type serine esterase (BLT-esterase) is found in light and dense granules fractionated from CTL. When CTL encounter their target cells, BLT-esterase is exocytosed from intracellular granules and is found in supernatant fluids. Secretion of BLT-esterase from a CTL line is inhibited by clonotypic antibodies specific for the receptor of that line. A direct demonstration that receptor cross-linkage leads to granule exocytosis was provided by the finding that polymeric anti-receptor antibodies lead to efficient secretion in the absence of target cells. The demonstration of the exocytosis of the BLT-esterase strongly suggests that this proteolytic enzyme plays an important role in the cytolytic process. Furthermore, it provides a direct and quantitative measurement of the triggering events which initiate the cytolytic process (Takayama and Sitkovsky, LI/NIAID; Berrebi and Henkart, IB/NCI).

Chemical Characterization of the Tumor Specific Transplantation Antigen of the Guinea Pig L2C Leukemia

L2C leukemia cells possess a powerful tumor specific transplantation antigen (TSTA) demonstrated by immunization protection tests with irradiated tumor cells and with membrane extracts from L2C cells. Immunogenicity appears to require association with class II major histocompatibility complex molecules

since L2C variants which possess the TSTA but lack class II molecules are not immunogenic for tumor protection. Laboratory of Immunology scientists have undertaken the purification of the TSTA in an effort to determine the structural features of this molecule which contribute to its immunogenicity and in order to determine whether it represents a member of a more widely distributed class of molecules, immunity to which may be important in anti-tumor responses to other leukemic cells. During the past year, the purification procedure has been considerably simplified and substantial progress has been made. A butanol extract of L2C cells is treated with trichloroacetic acid and the soluble material has protective activity upon immunization. This material is then resolved by chromatofocusing, where the active material is found to have a pI of ~10. Analysis of this material by sodium dodecyl sulfate-polyacrylamide gel electrophoresis demonstrates a single molecular species with an Mr of ~12,000. Efforts to obtain an amino acid sequence of this purified molecule are now in progress. This work promises to lead to a clearer understanding of the molecular basis of anti-tumor immunity in this experimental leukemia (Gregg and Green, LI/NIAID; Maloy, LIG/NIAID; Hearing, LCB/NCI).

Honors, Award and Scientific Recognition

Laboratory of Immunology scientists play increasingly important roles in the international immunological community. During the past year, Dr. Paul was elected President of the American Association of Immunologists (AAI); Dr. Shevach was selected by the Council of the AAI as Editor-in-Chief designate of the Journal of Immunology, to assume the editorship in July of 1987; and Dr. Schwartz was the Chairman of the Gordon Research Conference on Immunology and Immunochemistry.

Laboratory scientists serve on editorial boards of many scholarly publications. In addition to his selection as Editor-in-Chief of the Journal of Immunology, Dr. Shevach is section editor for clinical immunology of the Journal of Immunology, is a member of the editorial boards of Cellular Immunology, the Journal of Immunologic Methods, and of the Proceedings for Experimental Biology and Medicine. He is a member of the advisory editorial board of the Journal of Molecular and Cellular Immunology. Dr. Paul is the editor of the Annual Review of Immunology and of the advanced textbook Fundamental Immunology. He is an advisory editor of the Journal of Experimental Medicine, an associate editor of Cell, a member of the editorial boards of Immunological Reviews and of the Journal of Molecular and Cellular Immunology and is consulting editor for immunology of the Cecil Textbook of Medicine. Dr. Schwartz is a member of the board of reviewing editors of Science and of the editorial boards of Immunology Today, the International Journal of Cell Cloning, and of the Journal of Molecular and Cellular Immunology. Dr. Germain is a member of the advisory editorial boards of The Journal of Molecular and Cellular Immunology, and of the Annales de L'Institut Pasteur. Dr. Mage serves on the editorial board of Immunogenetics. Dr. Green is a member of the editorial committee of Clinical Immunology and Immunopathology. Dr. Inman is an advisory editor of Molecular Immunology and is a member of the editorial board of Analytical Biochemistry. Dr. Chused is a member of the editorial board of Cytometry.

Dr. Paul was appointed to the Board on Basic Biology of the National Research Council (NRC), is a member of the NRC Committee on Research Opportunities in Biology, and is a member of the Board of Directors of the Federation of American Societies of Experimental Biology. He serves on the awards committee of the Lita Annenberg Hazen Awards for Excellence in Clinical Research and on the selection committee for the Alfred Sloan Prize. Dr. Paul is chairman of the Scientific Review Committee of the Cambridge Branch of the Ludwig Institute for Cancer Research and of the Advisory Committee of the Harold C. Simmons Arthritis Research Center at University of Texas Health Sciences Center at Dallas. He is a member of the Board of Scientific Consultants of the Memorial-Sloan Kettering Cancer Center, of the Board of Scientific Advisors of the Jane Coffin Childs Memorial

Fund for Medical Research, of the Scientific Advisory Council of the Cancer Research Institute, Inc., and of the Board of Directors of the Foundation for Advanced Education in the Sciences. Dr. Paul completed a term as chairman of the Scientific Board of Visitors of the Oklahoma Medical Research Foundation.

During the past year, Dr. Paul was coorganizer and an invited lecturer at the International Conference on Lymphocyte Activation and Immune Regulation held at Newport Beach, CA. He chaired a symposium and was a major speaker at the VI International Congress on Immunology. He gave major presentations at the Gordon Research Conference on Immunology and Immunochemistry, at the FASEB Summer Conference on Immunopharmacology, at the annual meeting of the Tissue Culture Association, and at the Christmas Lectures of the Illinois Science Lecture Association. He presented a Distinguished Faculty Lecture at the M. D. Anderson Hospital and Tumor Institute.

Dr. Shevach received a U.S.P.H.S. meritorious service medal. He chaired sessions and was an invited speaker at the Conference on the Immunology of Multiple Sclerosis and at the Gordon Research Conference on Immunology and Immunochemistry. He was an invited speaker at the U.S.-India Joint Conference on the Immunology of Leprosy and served as an instructor in the Biology of Parasitism course at the Woods Hole Marine Biology Institute.

In addition to chairing the Gordon Research Conference on Immunology and Immunochemistry, Dr. Schwartz delivered a plenary lecture at the annual meeting of the American Association of Immunologists and served as an advisor to the Howard Hughes Medical Institute-NIH medical student research program.

Dr. Germain was a session chairman and an invited speaker at the Gordon Research Conference on Immunology and Immunochemistry and at the Sixth Immune Response Gene Workshop. He gave major presentations at the Multiple Sclerosis Society Workshop on Genetic, Molecular and Cellular Aspects of Demyelinating Processes, at the Reticuloendothelial Society Workshop on Molecular Biology and Genetic Approaches in Leucocyte Biology, at the meeting of the U.S.-Japan Cooperative Program in Tumor Immunology, at the Arden House Symposium on Antigen Processing and Presentation, at the Mount Sinai Symposium on Immune Genes, and at the Fifth H2-HLA Cloning Workshop. He was the recipient of an NIH Director's Award.

Dr. Mage is the organizer of the FAES Immunology course and serves as AAI representative to the American Type Culture Collection. She gave invited lectures at the World Health Organization Course on the Immune System in Epalinges, Switzerland, was an American Society for Microbiology Foundation lecturer, and a speaker at the Federation of European Biochemical Societies course on "Genome Organization and Evolution".

Dr. Green served as a member of the NIAID Clinical Research subpanel and of the NIAID animal care committee. Dr. Inman was an invited lecturer at the Sixth International Symposium on Bioaffinity Chromatography and Related Techniques held in Prague, Czechoslovakia. Dr. Chused was a plenary lecturer at the meeting of the Society for Analytical Cytometry. Dr. Margulies was an invited lecturer at the FASEB Summer Conference on Autoimmunity and at the Gordon Research Conference on Immunology and Immunochemistry.

Dr. Mark Davis, a former post-doctoral fellow in the Laboratory of Immunology, received the Eli Lilly Award of the American Society for Microbiology for research partly carried on in the Laboratory of Immunology.

Administrative, Organizational and Other Changes

Dr. Thomas Chused joined the Laboratory of Immunology as a senior investigator, having transferred from the Laboratory of Microbial Immunity. In addition to his duties as a senior investigator, Dr. Chused will be responsible for NIAID intramural flow cytometry facility; he will supervise the operation of three flow cytometers. Dr. Yasuo Ishida and Ms. Elinor Brown transferred to the Laboratory of Immunology with Dr. Chused and will play major roles in his research program.

The Laboratory of Immunology is a major training center for young scientists. During the past year, Drs. Kurt Gunter, Kathryn Kimmel, Masanori Komatsu, Richard Kroczeck, Edmundo Lamoyi, Robert Lechler, Helen Quill, and Georg Stingl completed training periods in the Laboratory of Immunology. Each of these scientists made an important contribution to the research program of the Laboratory of Immunology.

Several post-doctoral fellows joined the Laboratory during the past year. They included Drs. N. Harindranath, Nicola Hole, Rosemarie Hunziker, Jonathan Mueller, Barbara Newman, Daniel Regnier, Jonathan Schneck, and Guido Trenn. In addition, Dr. Phillip Cohen, a faculty member in the Department of Medicine at the University of North Carolina School of Medicine, is taking a sabbatical year in the Laboratory of Immunology and Mr. Richard Lopez, a medical student at Stanford University School of Medicine, is working in the Laboratory of Immunology as part of the Howard Hughes Medical Institute-National Institutes of Health student research program.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00030-18 LI
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Antigen Recognition and Activation of Immunocompetent Cells		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: William E. Paul Chief LI, NIAID Others: M. Brown, Staff Fellow, LI, NIAID; J. Ohara, Visiting Associate, NIAID; J. Mizuguchi, Visiting Associate, LI, NIAID; W. Tsang, Medical Staff Fellow, LI, NIAID; P. Hornbeck, Guest Researcher, LI, NIAID; C. Snapper, Medical Staff Fellow, LI, NIAID; I. Green, Sr. Invest., LI, NIAID; D. Margulies, Invest., LI, NIAID; R. Germain, Sr. Invest., LI, NIAID; T. Chused, Sr. Invest., LI, NIAID; C. Peschel, Guest Researcher, LI, NIAID.		
COOPERATING UNITS (if any) J. Coligan & L. Maloy, LIR/NIAID; M. Beaven, LC/NHLBI; F. Finkelman & J. Mond, USUHS; R. Coffman & T. Mossman, DNAX; E. Vitetta, Univ. TX, Southwestern Med. Ctr; S. Morrison, Columbia Univ., College of P&S; L. Glimcher, Harvard Sch Public Health.		
LAB/BRANCH Laboratory of Immunology		
SECTION		
INSTITUTE AND LOCATION National Institute of Allergy & Infectious Diseases, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 7.9	PROFESSIONAL: 5.9	OTHER: 2.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The mechanisms regulating <u>antigen-recognition</u>, activation, cell cycle progression, proliferation, and differentiation of <u>lymphocytes</u> are key to the control of the cellular responses of the immune system. During the past year, we have examined the biochemical mechanisms through which receptor cross-linkage stimulates <u>B cell activation</u>. We have obtained evidence for regulation of signal transmission by <u>protein kinase C</u>. We have also shown that principal protein substrates of phosphorylation catalyzed by PKC in B lymphocytes are plasma membrane associated proteins several of which are also associated with cytoskeleton. In parallel with these studies, the functions of <u>B cell stimulatory factor-1</u> (BSF-1), a critical co-factor in B cell stimulation, have been evaluated. BSF-1 has been purified to homogeneity, an N terminal amino acid sequence has been obtained, and a <u>receptor-binding</u> assay has been developed. Functional studies indicate that BSF-1 is active on B and T cells and on other cell types of hematopoietic lineage, including mast cells and erythroid and megakaryocytic precursors. Finally, BSF-1 has been shown to play a critical role in <u>IgE expression</u> both in tissue culture and in animals. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00035-11 LI
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Specificity in Immune Responses		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: J. K. Inman Senior Investigator LI, NIAID		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Immunology		
SECTION		
INSTITUTE AND LOCATION National Institute of Allergy & Infectious Diseases, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 1.75	PROFESSIONAL: 1.0	OTHER: 0.75
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The principal aim of this project is to test the hypothesis of <u>general multispecificity</u> for the combining regions of <u>antibodies</u> and other kinds of <u>receptors</u>. Receptor sites, according to theory developed earlier in this project, should be capable of interacting with virtually any substance in a manner that will lower the standard free energy of the system and thus exhibit an equilibrium association constant greater than 1. Most associations will be weaker than ones commonly measured, but occasional substances may bind to a receptor with affinities high enough to affect biological function. Their structure may not necessarily resemble those of the recognized effector, substrate or antigen. </p> <p> The above hypothesis is being tested in the following way: Radiolabeled, monoclonal antibodies or solubilized receptors are passed through small, <u>affinity chromatography</u> columns. Accurate measurements are made of the retention (retardation) caused by a matrix-bound reference ligand in the presence and absence of many, diverse, suitably large compounds. The resulting retention values are employed directly in calculating <u>association constants</u> for these compounds and the receptor site. The distribution of affinities provides a description of the receptor's multispecific character. The technique of quantitative affinity chromatography, developed in this study, provides a general means for estimating very low to moderately strong association constants. </p> <p> Knowledge of multispecific interactions will be employed in re-evaluating general concepts of specificity in biological recognition. Special attention will be given to applying these findings to models of immune systems and control networks. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00036-21 LI
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Ig Genetics: Ontogeny and Differentiation of Cells of the Rabbit Immune System		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: R. G. Mage Others: N. McCartney-Francis E. Lamoyi E. Padlan G. H. Cohen T. Borsos A. Circolo	Senior Investigator Senior Investigator Visiting Associate Senior Investigator Senior Investigator Senior Investigator Visiting Associate	LI, NIAID LMI, NIDR LI, NIAID LMB, NIADDK LMB, NIADDK LIB, NCI LIB, NCI
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Immunology		
SECTION		
INSTITUTE AND LOCATION National Institute of Allergy & Infectious Diseases, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 3.0	PROFESSIONAL: 2.0	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)		
<p> We have used classical immunogenetic techniques as well as techniques of molecular biology to study the genetics of rabbit immunoglobulins (Igs) and T cell receptors and the regulated expression of the genes that encode these molecules. We are investigating why rabbits produce only trace amounts of Igs with light chains of the K2 isotype. We have now shown that mRNA encoding such light chains can be detected in splenocytes from rabbits infected with <i>Trypanosoma equiperdum</i> and at 100 to 1,000 fold higher levels in comparable mRNA preparations from Basilea rabbits. Basilea rabbits carry a mutation that results in loss of expression of the normal major Ig light chain type (K1b9). Recent studies revealed a mutation in J_K-C_K intron at the 3' RNA splice acceptor site (Lamoyi and Mage, 1985). In studies to further determine whether this is the explanation for the loss of K1 gene expression, we conducted S1 protection analyses and found small amounts of K1b9 mRNA in splenocytes from Basilea rabbits. Using intron and C_K probes we found that the relative proportions of processed and non-processed mRNAs differed greatly in preparations from Basilea compared to normal control rabbits. The results support the conclusion that abnormal and/or inefficient mRNA processing results in the Basilea phenotype (non-expression of K1b9 light chains). We have designed and used an oligonucleotide probe that can distinguish mRNA encoding K2 allotypic forms (bas1 and bas2) that differ by only a single base in the codon for amino acid 204. We are continuing to design and use this and other DNA probes to distinguish rabbit Ig and T cell receptor allotypes. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00147-11 LI
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) The Mechanism of Activation of Thymus-Derived Lymphocytes		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	R. H. Schwartz	Senior Investigator
Other:	M. Jenkins	Guest Worker
	H. Quill	Staff Fellow
	K. Ogasawara	Visiting Fellow
		LI, NIAID
		LI, NIAID
		LI, NIAID
		LI, NIAID
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Immunology		
SECTION		
INSTITUTE AND LOCATION National Institute of Allergy & Infectious Diseases, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	2.5	1
CHECK APPROPRIATE BOX(ES)		
<input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither		
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)		
<p>Two mechanisms were found for rendering <u>T cell clones</u> unresponsive in vitro. One involved stimulation with antigen and <u>antigen-presenting cells</u> fixed with a <u>chemical cross-linker</u>. The other involved stimulation with antigen and <u>Ia molecules in planar lipid membranes</u>. In both systems the T cells were rendered incapable of producing <u>interleukin 2 (IL-2)</u> when subsequently challenged with antigen and normal antigen-presenting cells, although the cells did express low levels of <u>IL-2 receptors</u> and <u>interleukin 3</u>. The nonresponsiveness lasted at least 8 days, suggesting that both systems may be useful in vitro models for <u>tolerance</u> induction.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00148-11 LI
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Immunological Studies of Guinea Pig L ₂ C Leukemia		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <div style="display: flex; justify-content: space-between;"> <div style="width: 30%;"> PI: I. Green Evan Gregg </div> <div style="width: 30%;"> Senior Investigator Visiting Fellow </div> <div style="width: 30%;"> LI, NIAID LI, NIAID </div> </div>		
COOPERATING UNITS (if any) LCB, NCI (V. Hearing); LIG, NIAID (L. Maloy); Medicine Branch, NCI (D. Longo & S. Bridges); Tenovus Research Laboratory, Southampton General Hospital, Southampton, England (F. Stevenson), Richard Youle, NINCDS, SNB.		
LAB/BRANCH Laboratory of Immunology		
SECTION		
INSTITUTE AND LOCATION National Institute of Allergy & Infectious Diseases, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 3.0	PROFESSIONAL: 2.0	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The L₂C leukemia is a B cell leukemia of inbred strain 2 guinea pigs. These cells have surface IgM and C3 receptors. Studies have shown that these leukemia cells possess a strong tumor specific transplantation antigen (TSTA) that can easily be demonstrated by immunization protection tests in syngeneic animals. A new procedure for extracting the TSTA has been employed using butanol extraction of the L₂C cells. The concentrated butanol extract is then precipitated with 5% trichloroacetic acid and the supernatant again concentrated and put onto a chromatofocusing column. A 12,000 m.w. fraction is obtained with a pI of 10 that is immunogenic; 12% PAGE indicates this material is composed of two closely placed bands. Amino acid composition and sequence analysis of this band are now in progress.</p> <p>In addition to the above, immunotherapeutic studies of the L₂C leukemia are being performed. First administration of a conjugate of ricin to a monoclonal antibody to the surface IgM idiotype produces a significant increase in life span in leukemia animals and about 30% of animals are cured. Second the administration of low dose interleukin-2 (IL-2) therapy produce significant increase in survival of leukemic animals. Both local and systemic administration of LAK cells and IL-2 also produced significant prolongation of life. However, in contrast to the results with immunotoxin, no long term cures were produced.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 A1 00186-13 LI						
PERIOD COVERED October 1, 1985 to September 30, 1986								
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Pathogenesis of Autoimmunity in Inbred Strains of Mice								
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: T. M. Chused</td> <td style="width: 33%;">Senior Investigator</td> <td style="width: 33%;">LI, NIAID</td> </tr> <tr> <td>Others: Elinor Brown</td> <td>Biologist</td> <td>LI, NIAID</td> </tr> </table>			PI: T. M. Chused	Senior Investigator	LI, NIAID	Others: Elinor Brown	Biologist	LI, NIAID
PI: T. M. Chused	Senior Investigator	LI, NIAID						
Others: Elinor Brown	Biologist	LI, NIAID						
COOPERATING UNITS (if any) Renu Lal, Visiting Fellow, LPD, NIAID; John Coligan, Section Chief, LIG, NIAID; Philip J. Baker, Section Chief, LMI, NIAID; Herb Cooper, Section Chief, NCI.								
LAB/BRANCH Laboratory of Immunology								
SECTION								
INSTITUTE AND LOCATION National Institute of Allergy & Infectious Diseases, NIH, Bethesda, MD 20892								
TOTAL MAN-YEARS: .6	PROFESSIONAL: .3	OTHER: .3						
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews								
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The genetic control and immunologic mechanisms of autoimmune disease is being investigated in the New Zealand strains of mice, their F₁ hybrids, and recombinant-inbred lines derived from them. We have found 1) that enlargement of Lyt-2⁺ T cells is significantly associated with the titer of anti-erythrocyte autoantibody and degree of hemolytic anemia; 2) T cell suppression is defective in old NZB mice; 3) NZB, and particularly the (NZB x NZW) F₁ hybrid, mice have a major resistance to induction of tolerance in the T cell¹ subpopulation; 4) Several abnormalities of proteins synthesized by lymphocytes from NZB mice can be demonstrated by two-dimensional gel electrophoresis. One, a 16 kd fragment of μ chain, is observed only in enlarged NZB B cells.</p>								

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00203-07 LI
PERIOD COVERED <u>October 1 1985 to September 30, 1986</u>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>Applications of flow cytometry</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: T. M. Chused Others: Linette Edison Jean McKay	Senior Investigator Biologist Biologist	LI, NIAID OSD, NIAID OSE, NIAID
COOPERATING UNITS (if any) B.J. Fowlkes Biologist LMI, NIAID		
LAB/BRANCH Laboratory of Immunology		
SECTION		
INSTITUTE AND LOCATION National Institute of Allergy & Infectious Diseases, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS 2.05	PROFESSIONAL: .3	OTHER: 1.75
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) <div style="text-align: center;"> <p><u>Flow cytometry is being used to analyze the differentiation of thymic lymphocytes. Instrumentation is being enhanced to allow for simultaneous four parameter immunofluorescence.</u></p> </div>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00223-05 LI																				
PERIOD COVERED October 1, 1985 to September 30, 1986																						
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Cellular Interactions in the Immune Response																						
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 40%;">R. H. Schwartz</td> <td style="width: 30%;">Senior Investigator</td> <td style="width: 20%;">LI, NIAID</td> </tr> <tr> <td>Others:</td> <td>D. Pardoll</td> <td>Medical Staff Fellow</td> <td>LI, NIAID</td> </tr> <tr> <td></td> <td>C. Hua</td> <td>Visiting Fellow</td> <td>LI, NIAID</td> </tr> <tr> <td></td> <td>B. Fox</td> <td>Guest Worker/Staff Fellow</td> <td>LI, NIAID</td> </tr> <tr> <td></td> <td>K. McCoy</td> <td>Guest Worker/Staff Fellow</td> <td>LI, NIAID</td> </tr> </table>			PI:	R. H. Schwartz	Senior Investigator	LI, NIAID	Others:	D. Pardoll	Medical Staff Fellow	LI, NIAID		C. Hua	Visiting Fellow	LI, NIAID		B. Fox	Guest Worker/Staff Fellow	LI, NIAID		K. McCoy	Guest Worker/Staff Fellow	LI, NIAID
PI:	R. H. Schwartz	Senior Investigator	LI, NIAID																			
Others:	D. Pardoll	Medical Staff Fellow	LI, NIAID																			
	C. Hua	Visiting Fellow	LI, NIAID																			
	B. Fox	Guest Worker/Staff Fellow	LI, NIAID																			
	K. McCoy	Guest Worker/Staff Fellow	LI, NIAID																			
COOPERATING UNITS (if any) LMI, NIAID, B. J. Fowlkes, R. Asofsky, and BRMP; NCI, A. Kruisbeek; LI, R. N. Germain																						
LAB/BRANCH Laboratory of Immunology																						
SECTION																						
INSTITUTE AND LOCATION National Institute of Allergy & Infectious Diseases, NIH, Bethesda, MD 20892																						
TOTAL MAN-YEARS: 3.0	PROFESSIONAL: 2.0	OTHER: 1																				
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews																						
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> We have applied the technique of <u>in situ hybridization</u> with probes specific for mRNA encoding the α, β, and γ chains of the T cell antigen-receptor, <u>interleukin 2</u> and the <u>interleukin 2 receptor</u>, to explore early molecular events in fetal thymocyte development. Our results show that the γ chain locus turns on prior to the β chain locus, that day 13-15 thymocytes make IL-2 and that differentiation in vitro of day 14 fetal thymocytes is associated with expression of α chain mRNA and the <u>Lyt2</u> cell surface molecule, without <u>L3T4</u> expression. </p>																						

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER <div style="border: 1px solid black; padding: 2px; text-align: center;">ZOI AI 00224-05 LI</div>																																
PERIOD COVERED <div style="border: 1px solid black; padding: 2px;">October 1, 1985 to September 30, 1986</div>																																		
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.) <div style="border: 1px solid black; padding: 2px;">Monoclonal Antibodies as Probes for T Cell Activation</div>																																		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 45%;">E. M. Shevach</td> <td style="width: 40%;">Senior Investigator</td> <td style="width: 20%;">LI, NIAID</td> </tr> <tr> <td>Others:</td> <td>K. G. Gunter</td> <td>Medical Staff Fellow</td> <td>LI, NIAID</td> </tr> <tr> <td></td> <td>G. Perreira</td> <td>Visiting Fellow</td> <td>LI, NIAID</td> </tr> <tr> <td></td> <td>R. Krocze</td> <td>Guest Worker/Visiting Fellow</td> <td>LI, NIAID</td> </tr> <tr> <td></td> <td>K. Kimmel</td> <td>Guest Worker</td> <td>LI, NIAID</td> </tr> <tr> <td></td> <td>D. Presky</td> <td>Guest Worker</td> <td>LI, NIAID</td> </tr> <tr> <td></td> <td>W. Yokoyama</td> <td>Guest Worker</td> <td>LI, NIAID</td> </tr> <tr> <td></td> <td>G. Stingl</td> <td>Guest Worker</td> <td>LI, NIAID</td> </tr> </table>			PI:	E. M. Shevach	Senior Investigator	LI, NIAID	Others:	K. G. Gunter	Medical Staff Fellow	LI, NIAID		G. Perreira	Visiting Fellow	LI, NIAID		R. Krocze	Guest Worker/Visiting Fellow	LI, NIAID		K. Kimmel	Guest Worker	LI, NIAID		D. Presky	Guest Worker	LI, NIAID		W. Yokoyama	Guest Worker	LI, NIAID		G. Stingl	Guest Worker	LI, NIAID
PI:	E. M. Shevach	Senior Investigator	LI, NIAID																															
Others:	K. G. Gunter	Medical Staff Fellow	LI, NIAID																															
	G. Perreira	Visiting Fellow	LI, NIAID																															
	R. Krocze	Guest Worker/Visiting Fellow	LI, NIAID																															
	K. Kimmel	Guest Worker	LI, NIAID																															
	D. Presky	Guest Worker	LI, NIAID																															
	W. Yokoyama	Guest Worker	LI, NIAID																															
	G. Stingl	Guest Worker	LI, NIAID																															
COOPERATING UNITS (if any) <div style="border: 1px solid black; height: 40px;"></div>																																		
LAB/BRANCH <div style="border: 1px solid black; padding: 2px;">Laboratory of Immunology</div>																																		
SECTION <div style="border: 1px solid black; height: 20px;"></div>																																		
INSTITUTE AND LOCATION <div style="border: 1px solid black; padding: 2px;">National Institute of Allergy & Infectious Diseases, NIH, Bethesda, MD 20892</div>																																		
TOTAL MAN-YEARS <div style="border: 1px solid black; padding: 2px; text-align: center;">5.6</div>	PROFESSIONAL <div style="border: 1px solid black; padding: 2px; text-align: center;">3.1</div>	OTHER <div style="border: 1px solid black; padding: 2px; text-align: center;">2.5</div>																																
CHECK APPROPRIATE BOX(ES) <table style="width: 100%;"> <tr> <td><input type="checkbox"/> (a) Human subjects</td> <td><input checked="" type="checkbox"/> (b) Human tissues</td> <td><input type="checkbox"/> (c) Neither</td> </tr> <tr> <td><input type="checkbox"/> (a1) Minors</td> <td></td> <td></td> </tr> <tr> <td><input type="checkbox"/> (a2) Interviews</td> <td></td> <td></td> </tr> </table>			<input type="checkbox"/> (a) Human subjects	<input checked="" type="checkbox"/> (b) Human tissues	<input type="checkbox"/> (c) Neither	<input type="checkbox"/> (a1) Minors			<input type="checkbox"/> (a2) Interviews																									
<input type="checkbox"/> (a) Human subjects	<input checked="" type="checkbox"/> (b) Human tissues	<input type="checkbox"/> (c) Neither																																
<input type="checkbox"/> (a1) Minors																																		
<input type="checkbox"/> (a2) Interviews																																		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <div style="border: 1px solid black; padding: 10px; min-height: 300px;"> <p>The major objective of our studies is to characterize cell surface structures on T and B lymphocytes as well as on non-T accessory cells (AC) which, in addition to the specific antigen receptor, are involved in the process of lymphocyte activation. Over the past 5 years we have developed a number of monoclonal antibodies (mAbs) to mouse T lymphocyte cell surface antigens which are capable of stimulating or inhibiting T cell triggering. We have defined the Thy-1 antigen as one of the critical signal transducing molecules in lymphoid cells. A large panel of mAbs to Thy-1 were capable of inducing T cell activation. Furthermore, following transfection of Thy-1 into several murine B lymphomas, crosslinking of Thy-1 resulted in an elevation of cytoplasmic free calcium $[Ca^{2+}]_i$, one of the initial critical events in lymphocyte activation. Studies have also implicated a distinct group of cell surface alloantigens, the products of the Ly-6 locus, as important molecules involved in the physiological activation of T cells. Lastly, we have combined studies with mAbs to cell surface antigens with molecular studies of T cell receptor gene expression to characterize a unique population of T cells, the Thy-1 dendritic cells, which populate murine epidermis. The ultimate goals of our studies are to fully understand the regulatory mechanisms that control T cell activation and differentiation. mAbs to lymphocyte surface antigens should prove to be useful tools in these studies and may also prove to be attractive candidates for in vivo therapeutic use in attempts to modulate or abrogate an ongoing immune response.</p> </div>																																		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00226-05 LI
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Rabbit Allotypes: Structure, Organization and Regulated Expression of Ig Genes		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: R. G. Mage Others: E. Lamoyi M. Komatsu B. Newman N. Hole N. Harindranath	Senior Investigator Visiting Associate Guest Researcher (to 2/86) Guest Researcher (from 1/86) Visiting Fellow (from 3/86) Visiting Fellow (from 5/86)	LI, NIAID LI, NIAID LI, NIAID LI, NIAID LI, NIAID LI, NIAID
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Immunology		
SECTION		
INSTITUTE AND LOCATION National Institute of Allergy & Infectious Diseases, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 2.8	PROFESSIONAL: 1.8	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> We are conducting studies of genes of the rabbit immune system by techniques of <u>molecular biology</u>. We have demonstrated <u>evolutionary conservation</u> of both sequence and linkage relationships of gene segments encoding <u>T cell receptor β chain</u> variable regions. Three V_β genes, each represented as only a single copy in the haploid genome of the rabbit was found on a 4,291 bp genomic DNA fragment. They do not appear to have classical upstream promoter sequences but all three are found expressed as mature size mRNA transcripts. The linked rabbit V_β are remarkably different from each other and are more similar to human and mouse V_β than to each other. This suggests that the genes duplicated early, prior to rabbit-mouse radiation. We have discovered restriction fragment length polymorphism (RFLP) of the rabbit T cell receptor β chain constant region genes as well as probable allotypic forms. We are using the RFLP and allotypes to study whether rabbit C_β and C_κ are genetically linked as they are in mice, or unlinked as in man. A germline V_β gene with homology to mouse V_β 86T1 had a DNA sequence identical to that of the V_β portion of a cDNA from a second rabbit suggesting that the expressed gene had not undergone any <u>somatic mutations</u> (Lamoyi, Angiolillo and Mage, 1986). Like other species, rabbits have <u>two similar C_β genes</u> but we have now shown that some rabbits appear to have allotypic forms of C_β1 and three different C_β. Some C_β allotypic differences are found at amino acid positions where analogous C_κ allotypic differences occur. </p>		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00229-04 LI

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies on Lymphocyte Differentiation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	W. E. Paul	Chief	LI, NIAID
Others:	D. Cohen	Medical Staff Fellow	LI, NIAID
	E. Nielsen	Biologist	LI, NIAID

COOPERATING UNITS (if any) Dept Med. Microbiology, Stanford Univ. School of Medicine, Stanford, CA (M. Davis); Dept Biology, Univ. California-San Diego (S. Hedrick); A+R, NIADDKD (J. Siegel and A. Steinberg), and MET, NCI (T. Waldmann).

LAB/BRANCH

Laboratory of Immunology

SECTION

INSTITUTE AND LOCATION

National Institute of Allergy & Infectious Diseases, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

PROFESSIONAL

OTHER

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

This project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00259-04 LI

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

1a Molecules and Immune Response Genes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	W. E. Paul	Chief	LI, NIAID
Others:	M. Brown	Guest Researcher	LI, NIAID
	R. N. Germain	Senior Investigator	LI, NIAID

COOPERATING UNITS (if any)

Harvard School of Public Health, Boston, MA (L. Glimcher)

LAB/BRANCH

Laboratory of Immunology

SECTION

INSTITUTE AND LOCATION

National Institute of Allergy & Infectious Diseases, Bethesda, MD 20892

TOTAL MAN-YEARS

PROFESSIONAL

OTHER

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

This project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00349-04 LI
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Structure and Function of Murine Class II MHC Genes and Gene Products		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: R. Germain Senior Investigator LI, NIAID Others: N. Braunstein, Medical Staff Fellow, LI, NIAID; A. Sant, Guest Worker, LI, NIAID; J. Miller, Guest Worker, LI, NIAID; F. Ronchese, Visiting Fellow, LI, NIAID; R. Lechler, Guest Worker, LI, NIAID; J. Tou, Res. Technician, LI, NIAID; D. Margulies, Senior Staff Fellow, LI, NIAID; J. McCluskey, Guest Worker, LI, NIAID.		
COOPERATING UNITS (if any) LI, NIAID (E. Long); Dept Biochemistry, Harvard Univ., Cambridge, MA (J. Strominger); IB, NCI (A. Singer).		
LAB/BRANCH Laboratory of Immunology		
SECTION		
INSTITUTE AND LOCATION National Institute of Allergy & Infectious Diseases, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS	3.5	PROFESSIONAL: 2.5 OTHER: 1.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Class II MHC (Ia) gene products play critical roles in a variety of T lymphocyte responses. A combination of immunological and molecular genetic approaches is being used to gain an understanding of the relationship between Ia structure and function.</p> <p>The expression of Ia molecules has been examined at the protein level following transfection of class II α and β genes into murine L cells (fibroblasts). These studies reveal an unexpected pattern to the cell surface Ia expression obtained using different allelic α and β genes. Genes carried on the same chromosome give more efficient expression than those on opposite chromosomes, indicating that the α and β genes have undergone a process of co-evolution to maintain compatibility for expression. The region controlling this effect has been mapped to the first 50 amino acids of the β chain. Similar transfection studies using genes from different subregion (I-A vs I-E) show that allelically conserved carboxy-terminal regions of the β chain play no role in selective $\alpha\beta$ chain pairing, with the same amino-terminal β1 segment involved in intralocus pairing again playing the predominant role.</p> <p>The effects of variation in $\alpha\beta$ allelic origin, or in particular residues of the β chain, on antibody and T cell recognition of Ia indicate that the polymorphic regions of these chains interact to create new conformations seen by antibodies and T cell receptors. This result makes it difficult to create a physical map of the functional regions of Ia using only the mutagenesis approach, and indirect assays like T cell activation.</p> <p>These studies provide new insight into the evolution of histocompatibility molecules, the general processes of intracellular protein assembly and transport, and the control of T cell responses by structural variation in MHC molecules.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00394-03 LI

PERIOD COVERED

October 1 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Genetic Analysis of Lymphocyte Function

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	D. H. Margulies	Senior Investigator	LI, NIAID
Others:	J. McCluskey	Visiting Scientist	LI, NIAID
	L. Boyd	Chemist	LI, NIAID
	A. Ramsey	Guest Worker	LI, NIAID
	R. Lopez	Guest Worker	LI, NIAID

COOPERATING UNITS (If any)

LI, NIAID (R. Germain & J. Inman); LIG, NIAID (A. Lew & J. Coligan);
IB, NCI (J. Bluestone & A. Singer); LMB, NIADDK (D. Davies & E. Padlan).

LAB/BRANCH

Laboratory of Immunology

SECTION

INSTITUTE AND LOCATION

National Institute of Allergy & Infectious Diseases, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

3.0

PROFESSIONAL:

2.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The ongoing focus of the laboratory is to understand in detail the structure/function relationships of cell surface molecules involved in the the immune response. In particular we have made significant progress over the past year in five areas: A. Using molecular engineering techniques to generate genes capable of encoding soluble murine class I major histocompatibility complex (MHC) antigens; B. Using the purified soluble antigens derived from these cells to analyse the biochemical and functional behavior of soluble H-2D^d; C. Analyzing the origin and function of proteins derived from alternatively spliced mRNAs of the class I antigens H-2D^d and H-2K^d; D. Analyzing the structure-function relationship of class I molecules by a detailed serological, biochemical and functional study of H-2D^d/H-2L chimeric proteins; E. Studying the function of in vitro class II/class I chimeric genes.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00400-02 LI

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunoregulatory Disorders in Systemic Lupus Erythematosus

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	I. Green	Senior Investigator	LI, NIAID
Others:	H. Suzuki	Visiting Fellow	LI, NIAID
	K. Nakanishi	Visiting Fellow	LI, NIAID

COOPERATING UNITS (if any)

Arthritis Branch, NIADDK (A. Steinberg)

LAB/BRANCH

Laboratory of Immunology

SECTION

INSTITUTE AND LOCATION

National Institute of Allergy & Infectious Diseases, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

PROFESSIONAL

OTHER

CHECK APPROPRIATE BOX(ES)

- | | | |
|---|---|--------------------------------------|
| <input type="checkbox"/> (a) Human subjects | <input checked="" type="checkbox"/> (b) Human tissues | <input type="checkbox"/> (c) Neither |
| <input type="checkbox"/> (a1) Minors | | |
| <input type="checkbox"/> (a2) Interviews | | |

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00403-03 LI
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Molecular Genetic Analysis of T Cell Receptor Structure and Repertoire		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: R. N. Germain Senior Investigator LI, NIAID Others: T. Saito, Visiting Fellow, LI, NIAID; M. Avigan, Medical Staff Fellow, LI, NIAID; R. Lechler, Guest Worker, LI, NIAID; R. Schwartz, Senior Investigator, LI, NIAID; D. Pardoll, Medical Staff Fellow, LI, NIAID; E. Shevach, Senior Investigator, LI, NIAID; K. Gunter, Medical Staff Fellow, LI, NIAID; R. Kroczeck, Guest Worker, LI, NIAID; G. Stingl, Guest Worker, LI, NIAID		
COOPERATING UNITS (if any) LMI, NIAID (B. J. Fowlkes); Medicine Branch, NCI (A. Kruisbeek)		
LAB/BRANCH Laboratory of Immunology		
SECTION		
INSTITUTE AND LOCATION National Institute of Allergy & Infectious Diseases, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 3.5	PROFESSIONAL: 2.5	OTHER: 2.5
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> <u>T lymphocytes</u> divide and differentiate when they receive an appropriate activation signal. This signal can be provided by a clonally-distributed, specific antigen receptor, or through certain broadly distributed cell membrane structures. <u>Molecular biological</u> and in vitro cell culture techniques have been used to investigate the expression of the genes encoding these activation structures, and to study the relationship between structure and function. </p> <p> Direct evidence was obtained that the $\alpha\beta$ heterodimer identified on T cells by anti-clonotypic antibodies completely determines the specificity of a given T lymphocyte. This was accomplished by <u>DNA-mediated gene transfer</u> of α and β genes into human T cells, which acquired the specificity of the mouse T cell donating the genes. Similar gene transfer experiments using the <u>Thy-1 gene</u> suggest that the activation function of this non-clonally distributed T cell marker may be related to the T3-dependent triggering mechanism used by the $\alpha\beta$ receptor. </p> <p> Ontogenetic studies were carried out using <u>in situ hybridization</u> methods involving specific radioactive RNA probes. These experiments revealed the ordered expression of T cell receptor γ, β, then α genes during fetal development of T cells in the thymus. They also showed the acquisition and loss of high levels of interleukin-2 mRNA and interleukin-2 receptor mRNA in the developing T cell population. RNA and DNA analysis of an unusual Thy-1 positive cell from the skin revealed that these cells expressed T cell receptor genes in patterns resembling these different stages of thymic T cell development. </p> <p> These studies will enhance our knowledge of which cell membrane molecules are involved in triggering T cells to exert regulatory and effector function, and our understanding of the structural basis for specific T cell responses to antigen. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 A1 00425-02 L1
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Lymphocyte Physiology		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	T. M. Chused	Senior Investigator LI, NIAID
Others:	H. A. Wilson	Guest Worker LMI, NIAID
	D. Greenblatt	Medical Officer LMI, NIAID
	Y. Ishida	Visiting Fellow LI, NIAID
	Y.Y. Ji	Visiting Fellow LMI, NIAID
	Linette Edison	Biologist OSD, NIAID
	Elinor Brown	Biologist LI, NIAID
COOPERATING UNITS (if any) W. E. Paul, Chief, LI, NIAID; Larry Samelson, Senior Staff Fellow, CBMB, NICHD; Richard Klausner, Chief, CBMB, NICHD; Roger Tsien, Associate Professor, U. C. Berkeley; Fred Finkelman, Professor, Dept. Med., USUHS		
LAB/BRANCH Laboratory of Immunology		
SECTION		
INSTITUTE AND LOCATION National Institute of Allergy & Infectious Diseases, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.1	1.4	0.7
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.) <p>The process of <u>signal transduction</u> across the lymphocyte plasma membrane is under investigation. Novel <u>fluorescent probes</u> of physiologic parameters such as <u>membrane potential</u>, <u>intracellular pH</u> and <u>intracellular calcium</u>, in conjunction with the high sensitivity and single cell resolution of <u>flow cytometry</u>, are being utilized.</p> <p>We have found that positively charged cyanine dyes are not suitable for measuring membrane potential in cells containing mitochondria, but that negatively charged oxonol dyes are reliable indicators. Lymphocytes and monocytes, but not granulocytes, buffer membrane potential over more than the physiologic range of extracellular potassium ion. T lymphocytes, but not B lymphocytes, possess a calmodulin-dependent, calcium-sensitive potassium channel.</p> <p>A new calcium probe, indo-1, is very useful for measuring intracellular free calcium ion. We find that blocking the IgG Fc receptor of B cells significantly prolongs the calcium transient induced by anti-immunoglobulin.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00426-01 LI
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Organization of the Antigens in the Plasma Membranes of the Lymphocyte Clones		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	M. V. Sitkovsky	Visiting Scientist
		LI, NIAID
Others:	H. Takayama	Visiting Fellow
		LI, NIAID
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Immunology		
SECTION		
INSTITUTE AND LOCATION National Institute of Allergy & Infectious Diseases, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <div style="text-align: center; margin-top: 100px;"> This project has been terminated. </div>		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-A1 00427-02 L1

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Antigen-Specific and Antigen-Nonspecific Cellular Cytotoxicity

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Michail V. Sitkovsky	Visiting Scientists	LI, NIAID
Others:	Hajime Takayama	Visiting Fellow	LI, NIAID
	Guido Trenn	Guest Worker	LI, NIAID

COOPERATING UNITS (if any)

LCM, NHLBI (Dr. Randall Kincaid), and Immunology Branch,
NCI (Dr. Gabriel Berrebi, Dr. J. Bluestone and Dr. Pierre Henkart).

LAB/BRANCH

Laboratory of Immunology

SECTION

INSTITUTE AND LOCATION

National Institute of Allergy & Infectious Diseases, NIH, Bethesda, MD 20892

TOTAL MAN YEARS

3.0

PROFESSIONAL

2.0

OTHER

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

Attachment of cytotoxic T-lymphocytes (CTL) to Ag-bearing target cells (TC) results in the activation of CTL and in the destruction of TC. Molecular mechanisms of CTL-TC interactions are poorly understood. We attempted to identify proteins and enzymatic activities involved in CTL activation and effector functions. Several independent complementary approaches were used to address these questions. I. We found that protein kinase C activators and Ca⁺⁺ ionophores bypass the requirement for Ag-receptor mediated recognition by triggering conjugate formation and delivery of a lethal hit to non-Ag-bearing TC by murine CTL. These results appear to rule out the role of links between antigen and antigen receptor in conjugate formation and CTL activation and implicate protein kinase C and calcium increases in these CTL functions. II. In an attempt to clarify Ca⁺⁺-dependent reactions we have studied Ca⁺⁺-binding and Calmodulin (CaM)-binding proteins in lymphocytes in association with Dr. R. Kincaid. We found that the major CaM-binding protein in resting T- and B-lymphocytes is a protein phosphatase, "calcineurin". This result suggests the importance of reactions of dephosphorylation in "on" and "off" signalling in lymphocytes. III. We have demonstrated that "on" signals in CTL clones result in the exocytosis of the content of low pH intracellular granules and that this exocytosis is also regulated by protein kinase C and calcium. We have proposed a new general short term assay for the study of CTL activation. This assay is based on the detection of activity of a serine esterase, which is secreted rapidly in the supernatants in response to antigen-receptor crosslinking.



LABORATORY OF IMMUNOPATHOLOGY
1986 Annual Report
Table of Contents

<u>201 AI</u> <u>Project Number</u>		<u>Page</u>
Summary		6-1
00013-23	Immunobiology and Pathogenesis of DNA Virus Infections -- Lewis	6-6
00135-12	Properties of Immunoglobulin Secreting Cells -- Morse	6-7
00138-12	Viruses and Immune Response -- Morse	6-8
00205-06	Abnormalities of T and B Lymphocytes of Autoimmune Mice -- Morse	6-9
00284-05	Characterization of Pathogenic Murine Leukemia Viruses -- Hartley	6-10
00286-05	Studies of Genetic Control of Murine Leukemia Viruses and Virus-Induced Neoplasms -- Hartley	6-11
00465-01	Retrovirus-induced Murine Immunodeficiency Syndrome -- Morse	6-12



PHS-NIH
SUMMARY REPORT

ANNUAL REPORT OF THE LABORATORY OF IMMUNOPATHOLOGY, NIAID
October 1, 1985 to September 30, 1986

Herbert C. Morse III, M.D.
Chief, Laboratory of Immunopathology

This report marks the first anniversary of the formation of the Laboratory of Immunopathology. The year has been highly productive as the result of extensive collaborative studies within the Laboratory and continuing interactions with a large number of investigators at the NIH and other institutions. The focus of most of these studies has been on the mechanisms by which retroviruses and adenoviruses induce disease. A wide variety of approaches have been employed to a) evaluate the genetic organization of viruses in relation to their pathogenic properties, b) define the characteristics of normal target cell populations and the effects of viruses on these cells, and c) identify host characteristics that influence the outcome of virus infections. The application of molecular technology to various aspects of these studies has increased significantly over the last year although in vivo analyses of viral pathogenicity in mice and hamsters and immunologic studies of cells from normal, mutant and virus-infected mice still form the foundation of the Laboratory's efforts.

I would like to draw particular attention in this part of the summary to one aspect of our studies that exemplifies the strengths of combined virologic and immunologic approaches to studying the pathogenesis of a murine disease that may have profound implications for understanding host-virus interactions in humans infected with human immunodeficiency viruses. This work was begun three years ago to determine if retroviruses, termed LP-BM5 MuLV, that induce polyclonal B cell activation in mice could be used in vitro to generate immortalized polyclonal B cells for studies of signals inducing B cell growth and differentiation. Although these expectations proved to be unfounded, studies of mice infected with these viruses revealed that susceptible strains have many immunologic abnormalities in common with AIDS patients. These include a) polyclonal B activation associated with enhanced secretion of immunoglobulins and hypergammaglobulinemia, b) loss of T and B cell proliferative responses to mitogens and T cell proliferative and cytotoxic (CTL) responses to alloantigens, and c) failure of B cells to generate antigen-specific responses or to respond to normal T cell help (Mosier, Yetter, Morse). More recently, it was found that infected mice lose CTL reactivity to "self plus x" before CTL responses to alloantigens but that these responses can be restored by addition of exogenous IL-2 (Yetter, Shearer, Morse). Failure to generate

responses to "self plus x" was correlated with abrogation of resistance to an orthopox virus infection that causes no disease in normal mice (Buller, Yetter, Morse). Mice infected with these viruses also have markedly reduced NK activity although LAK function can be induced with IL-2 (Wunderlich, Yetter, Morse). Finally, some infected mice die with B cell lymphomas with brain involvement (Yetter, Fredrickson, Klinken, Hartley, Morse). The parallels between these findings and those reported for AIDS patients are obvious and striking.

From viral pathogenesis studies, we have found that the distribution of sensitivity of inbred strains to disease induced by the B-tropic LP-BM5 virus complex gives a pattern not predictable by known host genetic control mechanisms such as Fv-1 and Rmcf. Disease occurs in some but not all Fv-1^b strains, in certain Fv-1^a strains and in (C57BL/6 x CBA/N)F₁ and the reciprocal cross, hybrids which would be expected to be highly resistant by virtue of their Fv-1^{nb} Rmcf^{rs} character (Yetter, Mosier, Hartley, Morse). Interesting differences in onset and progression of the disease as well as some anomalies of virus replication have been observed (Hartley, Yetter, Morse). Further studies of this unique murine system may prove to be of great value in understanding the mechanisms involved in retrovirus-induced immunosuppression and genetically-determined variations in response to retroviral infections in both mouse and man.

Other highlights of the year's activities are as follows:

Molecular characterization of a helper-independent lymphomagenic MCF virus. An unusual MCF MuLV which efficiently induces T-cell lymphoma in NFS mice without requirement for ecotropic virus has been molecularly cloned. Two infectious DNA clones have been partially sequenced, one with high lymphoma inducing ability and one which induces disease with lower frequency, longer latent period, and more variable phenotype. The most striking difference between the highly active and the poorly lymphomagenic clone is that the former possesses in its LTR a 53 nucleotide duplication, which includes the enhancer region (Chattopadhyay, Baroudi, Hartley, Morse).

LTR sequences determine leukemogenicity of Moloney and Friend MuLV. A small number of nucleotide differences in the enhancer region of the LTR determines the ability of Friend MuLV to induce erythroleukemia, or Moloney MuLV to induce lymphoblastic lymphoma. Insertion of a fragment of 171 bases, including only the direct repeat sequences of Moloney virus, was sufficient to convert Friend MuLV to a virus which induced primarily lymphomas, while the substitution in Moloney virus of the direct repeat plus a short GC rich region just 3' of the enhancer from Friend MuLV resulted in a virus inducing only erythroleukemia (Hartley, Hopkins, Fredrickson).

Moloney and Friend MuLVs possessing only a single copy of the transcriptional enhancer sequences replicate efficiently in mice

and induce disease characteristic of the parental virus but with extended latent period (Hartley, Hopkins).

Phenylhydrazine stimulates formation of hematopoietic stem cells. The strong age dependent resistance of mice to pre B-cell lymphoma induction by the NS-1 defective transforming virus can be abrogated by selection of appropriate helper MuLV, use of intravenous injection, and pretreatment of mice with phenylhydrazine. These findings provide a means of comparing the pathogenicity of this apparently unique transforming virus with oncogenes such as v-Abl which are active in adult mice (Klinken, Morse).

Common abnormalities of lpr and gld mutant mice. Studies of the relations between the abnormal T cells induced by the lpr and gld mutations and induction of autoimmunity have been hampered by the inability to study the abnormal cells in isolation. Using plate separation techniques, the abnormal cells from both mutations were specifically purified and characterized. The abnormal cells were shown to be of T cell origin, express the T cell receptor and to be identical for both lpr and gld in expression of cell surface antigens, lectin binding and functional studies indicating failure to respond to alloantigens by proliferation or generation of cytotoxic T cells. These results reinforce the suggestion that these mutations affect different enzymes in a common metabolic pathway (Davidson, Bedigian, Dumont, Fowlkes, Morse).

Expression of adenovirus E1A oncogene during cell transformation is sufficient to induce susceptibility to lysis by host inflammatory cells. Mammalian cells transformed by nononcogenic human adenoviruses exhibit high susceptibility to destruction by host mononuclear inflammatory cells. We have analyzed the viral gene regulation of the susceptibility of transformed cells to lysis by natural killer cells and activated macrophages. Comparisons of target cell lines transformed by overlapping segments of the adenovirus E1 transforming gene region that include the E1A 12S and 13S transcription units and the first of the two exons present in each of these E1A transcription units revealed that isolated expression of a single intact oncogene, E1A or each of its intact transcription units was sufficient to cause increased cytolytic susceptibility in the absence of detectable transformed cell surface expression or viral transplantation antigens and irrespective of histocompatibility antigen identity between killer cells and target cells. These results suggest that novel oncogene functions that are not linked to the expression of previously recognized cell surface target structures may actively induce the elimination of neoplastic cells by components of the host immune surveillance system (Cook, Walker, Lewis, Ruley, Graham, Pilder).

Tumorigenicity of hamster and mouse cells transformed by adenoviruses 2 and 5 is not influenced by the level of class I major histocompatibility antigens expressed on the cells.

Adenovirus 12 (Ad12) is considered to be a highly oncogenic virus because this virus induces tumors when injected into hamsters and mice and can transform normal rodent cells in tissue culture to cells that produce tumors when injected into immunoincompetent animals. Ad2 and Ad5 are classified as non-oncogenic viruses since they fail to induce tumors when injected into hamsters and mice. Both Ad2 and Ad5 can transform normal rodent cells in tissue culture to neoplastic cells that produce tumors only in immunocompetent animals. Data have been published suggesting that the E1A region of the Ad12 genome, but not the Ad5 genome, blocks the expression of class I histocompatibility antigens on the surfaces of transformed rat and mouse cells. The reduction in the amount of class I major histocompatibility antigen on the surface of Ad12 transformed cells would reduce a critical recognition signal for cytotoxic lymphocytes - the component of the cellular immune system that specifically recognizes and kills tumor cells based on dual recognition of foreign and class I histocompatibility antigens. The loss of class I histocompatibility antigen on cells transformed by highly oncogenic Ad12 as opposed to their presence on cells transformed by Ad2 and Ad5 has been proposed as a possible explanation for the differences in the oncogenicity of these Ad serotypes. By immunoprecipitation and flow cytometry, we have found that cells from 4 of 6 Ad2 and Ad5 transformed hamster and mouse lines expressed high levels of cell-surface class I major histocompatibility (MHC) antigens, while cells from 2 of these 6 lines expressed low levels of cell-surface class I antigens. The levels of class I MHC proteins expressed by cells from these latter 2 lines were comparable to the levels of cell-surface class I MHC proteins expressed by cells from Ad12 transformed hamster and mouse lines. The amounts of class I mRNA, analyzed by Northern blotting were, in general, consistent with the levels of class I antigens expressed on the surfaces of these cells. These results indicate that there is no correlation between the tumorigenicity in immunocompetent syngeneic adult rodents of Ad2 and Ad5 transformed hamster and mouse cells and the level of class I MHC antigens expressed in the surfaces of these cells. Thus the expression of different levels of class I MHC proteins does not seem to explain the differences in the oncogenicity between nononcogenic and highly oncogenic human Ad serotypes (Haddada, Lewis, Sogn, Coligan, Cook, Walker and Levine).

Relations between B cell and myeloid differentiation. Some murine tumors were found to simultaneously express characteristics usually restricted to normal cells within the B cell or myelomonocytic differentiation pathways. Analyses of single cell clones prepared from these tumors showed that cells with characteristics of mature macrophages derived from cells with phenotypes of early pre-B cells. These data indicate that these two pathways of hematopoietic differentiation may be closer than appreciated previously (Holmes, Pierce, Davidson, Bauer, Potter, Morse).

Induction of multiple neoplasms by retroviruses containing v-myc.
The role of deregulated myc expression in tumorigenesis was assessed by inoculating newborn mice with retroviruses containing avian v-myc. The mice developed a wide variety of neoplasms including pancreatic and mammary adenocarcinomas T cell, pre-B cell and B cell lymphomas and myelogenous leukemias. The time course of disease induction suggests that alterations in expression of other onc genes may be required to induce the fully transformed phenotype (Morse, Hartley, Fredrickson, Yetter, Cleveland, Rapp).

Awards, Name Lectures

Herbert C. Morse III, First Wallace P. Rowe Lecture, American Association of Laboratory Animal Science, October 1985.

Andrew M. Lewis, Jr., Invited address, University of Kentucky, Department of Medical Microbiology and Immunology, minisymposium on "NK Cell in Tumor Immunity", FASEB 1986 Meeting.

Andrew M. Lewis, Jr. and James L. Cook paper entitled "A new role for DNA virus early proteins in viral carcinogenesis", Science 227:15-20, 1985, selected for inclusion in Yearbook of Cancer.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00013-23 LIP
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Immunobiology and Pathogenesis of DNA Virus Infections		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI : A. M. Lewis, Jr. Others: J. A. Sogn J. Coligan H. Kulaga H. Haddada A. S. Levine C. T. Patch J. B. Bolen	Hd, Viral Pathogenesis Section Senior Investigator Senior Investigator Staff Fellow Visiting Fellow Scientific Director Senior Investigator Senior Staff Fellow	LIP, NIAID LIG, NIAID LIG, NIAID LIG, NIAID OSD, NICHD OSD, NICHD OSD, NICHD OSD, NICHD PB, DCT, NCI
COOPERATING UNITS (if any) National Jewish Center for Immunology and Respiratory Medicine, Denver, CO (J. L. Cook).		
LAB/BRANCH Laboratory of Immunopathology		
SECTION Viral Pathogenesis Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 5.0	PROFESSIONAL: 1.0	OTHER: 4.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Current thinking about the mechanism of carcinogenesis of human adenoviruses revolves around the capacity of different adenovirus serotypes to induce in infected or transformed cells the ability to succumb to or resist destruction by the specific (mediated by cytotoxic lymphocytes) or nonspecific (mediated by natural killer cells and macrophages) components of the cellular immune system. We have shown that the early genes of the nononcogenic human adenoviruses (Ad2, Ad5) induce in infected and transformed cells susceptibility to lysis by NK cells and macrophages while the early genes of highly oncogenic Ad12 induces either no susceptibility or induce resistance to NK cell lysis. Others have reported similar results and have also found that the Ad12 early genes interfere with the expression of class I major histocompatibility (MHC) antigens on the surface of Ad12 transformed rat and mouse cells thus removing a critical recognition signal for cytotoxic lymphocytes. We have now found the specific Ad2 and Ad5 early genes that induce susceptibility to NK cells and macrophages and have attempted to clarify the contribution toward tumorigenicity made by the expression of class I MHC antigens. This year we have identified the E1A oncogene of the nononcogenic Ad serotype as the gene that induces susceptibility to NK lysis and have found that both the E1A 12s and 13s transcription units can induce NK susceptibility. We have also found that Ad2 and Ad5 transformed mouse and hamster cells express levels of class I MHC antigens that are comparable to the levels present on Ad12 transformed cells. In spite of the loss of cell-surface class I antigens, the Ad2 and Ad5 transformed cells are unable to produce tumors in syngeneic adult hamsters and mice; Ad12 transformed cells from these species are highly tumorigenic in syngeneic adult animals. From these data it seems that there are barriers to tumorigenicity for cells transformed by Ad2 and Ad5 that are not relieved by reducing class I antigens and that Ad gene functions that govern the ability of transformed cells to be recognized and destroyed by NK cells and macrophages are among the primary determinants of the tumorigenicity of human Ad in rodents.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00135-12 LIP
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Properties of Immunoglobulin Secreting Cells		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI : H. C. Morse III Chief LIP, NIAID Others: W. F. Davidson Visiting Scientist LG, NCI J. W. Hartley Senior Investigator LIP, NIAID T. N. Fredrickson Research Microbiologist LIP, NIAID K. L. Holmes Staff Fellow LIP, NIAID S. P. Klinken Visiting Fellow LIP, NIAID U. R. Rapp Senior Investigator LVC, NCI M. Potter Chief LG, NCI		
COOPERATING UNITS (if any) DNAX Research Institute, Palo Alto, CA (R. L. Coffman); Sloan-Kettering Cancer Center, New York, NY (U. R. Hammerling).		
LAB/BRANCH Laboratory of Immunopathology		
SECTION Virology and Cellular Immunology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 2.0	PROFESSIONAL: 1.5	OTHER: 0.5
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> An unexpectedly wide variety of <u>oncogenes</u> have been found to contribute to the induction of murine B lineage lymphomas. Bone marrow or fetal liver cells transformed <u>in vitro</u> with retroviruses containing K-ras, H-ras, <u>bas</u>, <u>fes</u>, <u>src</u> and <u>erbB</u> were all shown to yield cell lines with characteristics of <u>pre-B cell lymphomas</u>. Detailed studies of some of these tumors and others indicated a close relationship between the B cell and myelomonocytic pathways of differentiation. Cells with many characteristics of mature macrophages were shown to be clonally related to cells with pre-B characteristics. </p> <p> Studies of tumors classified previously as plasmacytoid lymphosarcomas showed that they are <u>myelomonocytic</u> leukemias representing different stages of differentiation. Treatment of the cells with PMA resulted in maturation in the face of high levels of <u>myb</u> expressive. <u>Myb</u> expression was shown to be driven from the inserted retroviral LTRs. </p> <p> Newborn NFS mice inoculated with retroviruses containing an avian v-<u>myc</u> gene developed T cell, B cell lymphomas and mammary and pancreatic adenocarcinomas. The same viruses inoculated into adult pristane-primed BALB/c mice produced plasmacytomas and myelogenous leukemias. By comparison, treatment of pristane-primed mice with retroviruses containing a murine c-<u>myc</u> gene resulted exclusively in the development of myeloid tumors. </p>		

Others:	G. L. C. Shen	Investigator	LG, NCI
	S. R. Bauer	Investigator	LG, NCI
	J. H. Pierce	Investigator	LCMB, NCI
	J. F. Mushinski	Investigator	LG, NCI
	L. Wolff	Investigator	LG, NCI

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00138-12 LIP
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Viruses and Immune Response		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI : H. C. Morse III Chief LIP, NIAID Others: J. W. Hartley Senior Investigator LIP, NIAID K. L. Holmes Staff Fellow LIP, NIAID R. A. Yetter Guest Researcher LIP, NIAID T. N. Fredrickson Research Microbiologist LIP, NIAID R. M. L. Buller Staff Fellow LVD, NIAID J. N. Ihle Senior Investigator FCRC, NCI		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Immunopathology		
SECTION Virology and Cellular Immunology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 1.5	PROFESSIONAL: 1.5	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> Mice infected with a wild mouse-derived ecotropic murine leukemia virus (Cas-Br-M) develop a wide spectrum of <u>hematopoietic tumors</u>. To determine if these tumors were caused by the parental virus or newly-induced recombinant viruses with lineage specificity, mice were infected with extracts of primary tumors induced by Cas-Br-M. Two lineage-specific viruses were recovered. One, Cas NS-6 is a unique MCF virus that induces T cell lymphomas with short latency without the requirement for ecotropic virus. The second, Cas NS-1, is a replication defective virus that transforms fibroblasts <u>in vitro</u> and induces predominantly pre-B cell lymphomas <u>in vivo</u>. Additional studies of tumors induced by these extracts showed that <u>interleukin 3</u>-dependent cell lines with myeloid characteristics were obtained from myelogenous and erythroleukemias but rarely from lymphoid neoplasms. </p> <p> Resistance to <u>ectromelia virus</u> infection is determined by cytotoxic T lymphocyte responses. Studies of mice depleted <u>in vivo</u> of L3T4⁺ cells demonstrated that they produce normal responses to ectromelia and survive the infection. These results provide the first <u>in vivo</u> evidence for a helper cell-independent pathway for generation of cytotoxic T lymphocytes. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00205-06 LIP
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Abnormalities of T and B Lymphocytes of Autoimmune Mice		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI	: H. C. Morse III	Chief LIP, NIAID
Others:	W. F. Davidson	Visiting Scientist LG, NCI
	B. J. Fowlkes	Investigator LMI, NIAID
	E. K. Rudikoff	Bio. Lab. Tech. (Micro) LIP, NIAID
	L. Samelson	Investigator CBMB, NICHD
	R. Klausner	Investigator CBMB, NICHD
COOPERATING UNITS (if any) Jackson Laboratory, Bar Harbor, ME (H. R. Bedigian); Merck, Sharp and Dohme Research Laboratory, Rahway, NJ (F. J. Dumont).		
LAB/BRANCH Laboratory of Immunopathology		
SECTION Virology and Cellular Immunology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.75	0.25
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) <p> Mice bearing the non-allelic autosomal recessive mutations, <u>lpr</u> and <u>gld</u>, exhibit many abnormalities in common. All mouse strains homozygous for either of these mutations develop <u>lymphadenopathy</u>, <u>splenomegaly</u>, and <u>autoimmunity</u> of varying severity. These abnormalities appear to be due to expansion of an unusual subset of <u>T cells</u>. Methods were designed to specifically purify the abnormal T cells from mice with either mutation. The abnormal cells were shown to be of T cell origin and to express the <u>T cell receptor</u> on their surfaces. By a variety of criteria, the abnormal T cells from mice with either mutation were shown to be identical. In addition, an unusual intrinsic <u>phosphorylation</u> of a 21 KD protein was shared by the abnormal cells characteristic of both mutations. These findings reinforce the suggestion that <u>lpr</u> and <u>gld</u> may affect different enzymes in a common metabolic pathway of central importance to T cell function. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00284-05 LIP
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Characterization of Pathogenic Murine Leukemia Viruses		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI : J. W. Hartley Hd, Viral Oncology Section LIP, NIAID		
Others: H. C. Morse III Chief LIP, NIAID T. N. Fredrickson Research Microbiologist LIP, NIAID R. A. Yetter Guest Researcher LIP, NIAID K. L. Holmes Staff Fellow LIP, NIAID S. K. Chattopadhyay Guest Researcher LIP, NIAID C. Kozak Senior Investigator LMM, NIAID		
COOPERATING UNITS (if any) Massachusetts Institute of Technology, Cambridge, MA (Nancy Hopkins); Johns Hopkins, Baltimore, MD (Stephen Staal); Georgetown University, Washington, D.C. (Behige Baroudi).		
LAB/BRANCH Laboratory of Immunopathology		
SECTION Viral Oncology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 4.0	PROFESSIONAL: 2.3	OTHER: 1.7
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> This project is concerned with several aspects of investigations of the roles of replication competent murine leukemia viruses (MuLVs) in development of spontaneous or induced neoplastic and non-neoplastic disease in laboratory mice. Several classes of MuLVs are known, categorized on the basis of host range and ability to establish interference to superinfection, and pathogenic, biologic, or molecular variants within each class have been identified. Mice of selected strains are inoculated with various virus preparations, usually biologically cloned ecotropic or recombinant MCF MuLVs. Tests are carried out for replication of input virus and generation of new recombinant viruses, tumors or other lesions are studied histopathologically and when appropriate by surface antigen phenotyping, and may be examined for new proviral integrations, for rearrangements of cellular genes, or for expression of activated gene products. Insights into the molecular basis of pathogenicity and tissue specificity of disease are obtained by construction of recombinant viruses from molecular clones of related viruses with different pathogenic properties. Results of current studies include characterization of helper independent lymphomagenic MCF viruses, and the molecular cloning and partial sequencing of one of these; definition in Moloney (T-cell lymphomagenic) and Friend (erythroleukemia inducing) MuLVs those sequences responsible for disease specificity: the direct repeat region, having the properties of transcriptional enhancers; and recovery of new MuLV from California wild mice, the source of previous isolates with interesting pathogenic properties. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00286-05 LIP												
PERIOD COVERED October 1, 1985 to September 30, 1986														
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Studies of Genetic Control of Murine Leukemia Viruses and Virus-Induced Neoplasms														
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI : J. W. Hartley Hd, Viral Oncology Section LIP, NIAID														
Others: <table style="width: 100%; border: none;"> <tr> <td style="width: 40%;">H. C. Morse III</td> <td style="width: 40%;">Chief</td> <td style="width: 20%;">LIP, NIAID</td> </tr> <tr> <td>T. N. Fredrickson</td> <td>Research Microbiologist</td> <td>LIP, NIAID</td> </tr> <tr> <td>R. A. Yetter</td> <td>Guest Researcher</td> <td>LIP, NIAID</td> </tr> <tr> <td>S. P. Klinken</td> <td>Visiting Fellow</td> <td>LIP, NIAID</td> </tr> </table>			H. C. Morse III	Chief	LIP, NIAID	T. N. Fredrickson	Research Microbiologist	LIP, NIAID	R. A. Yetter	Guest Researcher	LIP, NIAID	S. P. Klinken	Visiting Fellow	LIP, NIAID
H. C. Morse III	Chief	LIP, NIAID												
T. N. Fredrickson	Research Microbiologist	LIP, NIAID												
R. A. Yetter	Guest Researcher	LIP, NIAID												
S. P. Klinken	Visiting Fellow	LIP, NIAID												
COOPERATING UNITS (if any) USC School of Medicine (Paul Pattengale); U. of Texas System Cancer Center (Frederick Becker).														
LAB/BRANCH Laboratory of Immunopathology														
SECTION Viral Oncology Section														
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892														
TOTAL MAN-YEARS: 3	PROFESSIONAL: 1.0	OTHER: 2.0												
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews														
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Genetic factors contributed by both host and virus interact to determine the effects of murine leukemia virus (MuLV) infection of mice. Host genes which influence MuLV-related hematopoietic system disease have been identified and chromosomally mapped; these include MuLV ecotropic virus induction loci, which represent integrated proviral sequences, and genes controlling infection and spread of virus, such as <u>Fv-1</u>, determining sensitivity to infection by N- or B-tropic MuLVs, and <u>Rmcf</u>, which affects replication and generation of MCF viruses. In order to study gene effects isolated from other host genetic differences, a number of strains of NFS mice congenic for different virus induction genes and for <u>Rmcf</u> from DBA/2 and CBA/N have been established or are being developed. In addition, ecotropic virus-free congenic lines of AKR/N, C57BL/10 and BALB/cn are being developed. V-congenic mice have been widely used in studies of spontaneous neoplastic disease, in oncogenicity studies of various acute transforming and replication competent MuLVs, and in studies of the relation of endogenous MuLV to carcinogen-induced tumors. Other host factors controlling MuLV-related disease are less well characterized and studies of these factors are still concerned with defining strain distribution and genetic patterns. In progress is a study of the genetics of disease induced by MuLV containing the oncogene <u>v-raf</u> or a construct with both <u>v-raf</u> and avian <u>v-myc</u> sequences. Also under study is the strain distribution of sensitivity to an unusual lymphoproliferative and immunosuppressive disease, in which known host gene controls appear not to function wholly as expected.</p>														

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00465-01 LIP																								
PERIOD COVERED October 1, 1985 to September 30, 1986																										
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Retrovirus-induced Murine Immunodeficiency Syndrome																										
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 30%;">PI : H. C. Morse III</td> <td style="width: 30%;">Chief</td> <td style="width: 40%;">LIP, NIAID</td> </tr> <tr> <td>Others: R. A. Yetter</td> <td>Guest Researcher</td> <td>LIP, NIAID</td> </tr> <tr> <td>J. W. Hartley</td> <td>Senior Investigator</td> <td>LIP, NIAID</td> </tr> <tr> <td>C. Shearer</td> <td>Investigator</td> <td>IB, NCI</td> </tr> <tr> <td>J. Wunderlich</td> <td>Investigator</td> <td>LIP, NIAID</td> </tr> <tr> <td>K. L. Holmes</td> <td>Staff Fellow</td> <td>LIP, NIAID</td> </tr> <tr> <td>S. P. Klitten</td> <td>Visiting Fellow</td> <td>LIP, NIAID</td> </tr> <tr> <td>S. K. Chattopadhyay</td> <td>Guest Researcher</td> <td>LIP, NIAID</td> </tr> </table>			PI : H. C. Morse III	Chief	LIP, NIAID	Others: R. A. Yetter	Guest Researcher	LIP, NIAID	J. W. Hartley	Senior Investigator	LIP, NIAID	C. Shearer	Investigator	IB, NCI	J. Wunderlich	Investigator	LIP, NIAID	K. L. Holmes	Staff Fellow	LIP, NIAID	S. P. Klitten	Visiting Fellow	LIP, NIAID	S. K. Chattopadhyay	Guest Researcher	LIP, NIAID
PI : H. C. Morse III	Chief	LIP, NIAID																								
Others: R. A. Yetter	Guest Researcher	LIP, NIAID																								
J. W. Hartley	Senior Investigator	LIP, NIAID																								
C. Shearer	Investigator	IB, NCI																								
J. Wunderlich	Investigator	LIP, NIAID																								
K. L. Holmes	Staff Fellow	LIP, NIAID																								
S. P. Klitten	Visiting Fellow	LIP, NIAID																								
S. K. Chattopadhyay	Guest Researcher	LIP, NIAID																								
COOPERATING UNITS (if any) Medical Biology Institute, LaJolla, CA (D. Mosier); Johns Hopkins Medical School, Baltimore, MD (P. Pitha).																										
LAB/BRANCH Laboratory of Immunopathology																										
SECTION Virology and Cellular Immunology Section																										
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892																										
TOTAL MAN-YEARS: 3	PROFESSIONAL: 2.5	OTHER: 0.5																								
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews																										
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> Previous studies of mice infected with LP-BM5 murine leukemia viruses showed that they exhibit many immunologic abnormalities described for <u>AIDS</u> patients infected with human <u>immunodeficiency</u> viruses. The current studies were developed to determine the pathogenesis of these defects in mice. In parallel with studies of man, it was found that cytotoxic T lymphocyte responses to "self plus x" antigens are lost before reactivity to allo-antigens. Studies of terminal mice showed that they died with B cell <u>lymphomas</u> with frequent brain involvement. Development of lymphomas was shown to progress from polyclonal B cell stimulation through oligoclonal proliferation to terminal neoplasia. Enhanced susceptibility to infection consequent to immuno-suppression was shown by the fact that C57BL/6 mice, normally resistant to ectromelia virus, die with ectromelia if previously exposed to Lp-BM5 viruses. Analyses of <u>lymphokine</u> production by cells of infected mice showed deficiencies in expression of IL-2, alpha, beta and gamma interferon. By comparison, expression of BSF-1 appears to be normal or perhaps increased. Finally mice infected with LP-BM5 MuLV show decreased NK activity. However NK or LAK activity and CTL responses can be restored by providing exogenous IL-2. These results suggest that studies of mice infected with LP-BM5 viruses may provide important insights into the mechanisms of immunosuppression induced by retroviruses. </p>																										

Others: R. M. L. Buller
T. N. Fredrickson
W. E. Paul

Staff Fellow
Research Microbiologist
Chief

LVD, NIAID
LIP, NIAID
LI, NIAID



LABORATORY OF IMMUNOREGULATION
1986 Annual Report
Table of Contents

<u>Z01-AI</u> <u>Project Number</u>		<u>Page</u>
Summary		7-1
00210-06	Immunoregulation of Human Lymphocyte Function in Normal and Disease States - Fauci	7-18
00212-06	Study of the Immunopathogenic Features of Immune-Mediated Diseases - Fauci	7-19
00213-06	Clinical, Immunopathogenic, and Therapeutic Studies in the Spectrum of Vasculitis - Fauci	7-20
00390-03	Studies of the Acquired Immunodeficiency Syndrome - Lane	7-21
00431-02	The Molecular Biologic Approach to the Immune System - Siebenlist	7-22
00361-04	International Studies of the Acquired Immunodeficiency Syndrome - Quinn	7-23
00358-04	Immunopathogenesis of <u>Chlamydia Trachomatis</u> Infection - Quinn	7-24



Summary Report
Laboratory of Immunoregulation
October 1, 1985 through September 30, 1986

Anthony S. Fauci, M.D.
Chief, Laboratory of Immunoregulation, NIAID

Studies of the Human B Cell Cycle: Mechanisms of Activation of Resting Human B Cells

Over previous years, we have developed a model system for the dissection of the events involved in the driving of human B cells from the resting state through proliferation and ultimately to terminal differentiation and antibody secretion. We have delineated the necessary and sufficient signals required to activate a resting B cell whereby it would become responsive to a variety of growth and differentiation factors. We have demonstrated that resting B cells can be activated via the crosslinking of their surface membrane immunoglobulin (sIg) molecules. This can be accomplished by a number of means which mimic specific antigen binding to sIg. Our model commonly employs anti-Ig or Staphylococcus aureus Cowan I (SAC) to crosslink sIg. Over the past year, we have investigated the mechanisms of direct activation of human B cells unrelated to sIg crosslinking. We have identified a B cell activating factor (BCAF) derived from a mitogenically stimulated T4+ human cell line and have purified it to homogeneity. It has a molecular weight (MW) of 11 kd and is clearly distinct from other previously described growth factors. It directly induces G₀ B cells to proliferate without inducing Ig secretion. In addition, we have identified a factor secreted by human T lymphotropic virus (HTLV)-I transformed T cells which directly activates a proportion of resting B cells to differentiate to Ig secretion. Finally, we have demonstrated that high molecular weight (HMW)-BCGF induced c-myc expression in resting B cells but did not directly induce proliferation or differentiation. This effect was specific for B cells and suggests along with other data that c-myc induction is one of the events involved in rendering a cell competent to proliferate. At the same time, this finding suggests that resting B cells do in fact have receptors for HMW-BCGF. HLA-unrestricted T cell-derived factors, which can transmit an activation signal to resting B cell independently of cell-cell contact or of antigen or mitogen, have important implications for the understanding of the polyclonal B cell hyperactivity of human autoimmune diseases and the mechanisms of abnormal B cell clonal expansion in vivo.

In other studies aimed at determining the T cell-B cell interactions involved in B cell activation, we employed the T cell-dependent system of pokeweed mitogen (PWM)-driven Ig production. We demonstrated that both the physical presence of T cells and the secretion of interleukin-2 (IL-2) were essential for the induction of Ig secretion in this system. T cells were necessary only in the initial stage of B cell triggering, whereas IL-2 was essential from the initial stage of B cell activation through the final stages of B cell differentiation. (Ambrus, Bowen, Kehrl, T. Nakagawa, N. Nakagawa, Siebenlist, Fauci, LIR/NIAID).

Studies of the Human B Cell Cycle: The Identification, Purification, and Characterization of Human B Cell Growth and Differentiation Factors and Their Cellular Receptors

Two years ago, we identified and isolated a HMW-BCGF produced by a human B cell lymphoma line as well as by a T cell lymphoma line. The factor had a MW of approximately 60 kd, and it preferentially enhanced the proliferation of normal human B cells which were already in the activated state and hence it provided a second signal for proliferation later on in the sequential steps of the B cell cycle. This factor was clearly distinguishable from other lymphokines known to enhance B cell proliferation, such as IL-1, IL-2, low molecular weight (LMW)-BCGF (which acts early in the B cell cycle), and the interferons. Over the past year, we have purified this HMW-BCGF to homogeneity, have developed a monoclonal antibody against it, and have demonstrated its specific binding to activated B cells. Indeed, we have identified and isolated the cellular receptor for the factor (see below). We have further demonstrated that the factor can be produced by normal T cells indicating that it may well play a physiologic role in the normal B cell cycle. Furthermore, we demonstrated that normal B cells produce a 32 kd BCGF, indicating a potentially important autocrine function of this factor. The HMW-BCGF has been partially sequenced, and studies are actively being pursued towards the cloning of the gene for this factor.

Over the past year, we have identified a protein which is or is associated with the cellular receptor for HMW-BCGF. Following the demonstration that HMW-BCGF binds specifically to activated B cells, we developed a monoclonal antibody, BA5, which blocked HMW-BCGF-driven B cell proliferation and preferentially bound to activated rather than resting B cells and did not bind to T cells. Crosslinking cell-bound HMW-BCGF to its cellular binding site together with immunoprecipitation studies using BA4 and BCGF/1/C2 (a monoclonal antibody to HMW-BCGF) demonstrated that the binding site for HMW-BCGF was a 90 kd protein which is recognized by BA5 antibody. In addition, BA5 protein has been successfully incorporated into liposomes and demonstrated to bind BA5 antibody and HMW-BCGF, but not IL-2 or BSA. While this confirms that BA5 protein contains a binding site for HMW-BCGF, it also allows further experiments to be done evaluating the nature of the binding of HMW-BCGF to its receptor, and the ability of BA5 protein transfected into non-B cells to bind HMW-BCGF and cause proliferation of the non-B cells.

In other studies, we have developed a panel of B cell clones derived from a B cell lymphoma line which share identical Ig gene rearrangements. Certain clones selectively produce or respond to BCGF. Preliminary studies characterizing the BCGF produced by these cloned lymphoma cells have shown that it is of LMW and elutes by reverse phase chromatography with thromboxane B2 (TXB2). While it is not clear that TXB2 is the only material present in the eluted BCGF peak, we have shown that purified BCGF enhances proliferation not only of responder clones but also of normal B cells. This raises the possibility that TXB2 and other prostaglandins (PG) may function either as first or second messengers in B cell proliferation and have other roles in normal B cell physiology.

With regard to B cell differentiation factors (BCDF), we have previously demonstrated that a T4+/Leu8+ T cell clone (YA2) selectively secreted BCDF, but not BCGF. This BCDF has a MW of 30 kd, a pI of 6-6.9, and induced Ig secretion by SAC-activated B cells and factor-responsive B cell lines. Over the past

year, we have purified this factor and have demonstrated selective binding of radiolabeled BCDF to SKW 6.4, a BCDF-responsive cell line and not to IL-2-responsive transformed B cell lines.

We had previously demonstrated that normal human B cells can be induced to express receptors for IL-2 and that they can proliferate and differentiate in response to recombinant IL-2. Over the past year, we have shown by competitive binding and blocking studies with an anti-IL-2 receptor monoclonal antibody that IL-2 reacts with receptors on B cells that are distinct from receptors for BCGF and BCDF. In addition, we have examined the expression of mRNA for IL-2 receptors on human B cells and have further confirmed that IL-2 is an important regulatory factor in human B cell function and have demonstrated a striking difference in the magnitude of induction of IL-2 receptors at the mRNA level between T cells and B cells. Finally, we have demonstrated that resting B cells express low numbers of high affinity receptors and that exposure to sufficient quantities of IL-2 results in a rapid upregulation in IL-2 receptor mRNA, IL-2 receptor expression, and entrance into S phase.

Over the past year, we have demonstrated that a number of factors not generally thought to have effects on the B cell cycle in fact have profound regulatory effects on human B cell function. We had previously demonstrated that transforming growth factor beta (TGF- β) suppressed factor-dependent T and B cell proliferation, B cell Ig secretion, and the cytolytic activity of large granular lymphocytes. Both T cells and B cells were shown to have high affinity TGF- β receptors, and mitogen activation resulted in a 4- to 5-fold increase in receptor number. We have recently demonstrated that B cells synthesize and secrete TGF- β in a concentration to sufficiently impair their own proliferation and Ig synthesis suggesting a role for TGF- β as an autocrine regulator of B cell function. Since TGF- β is known to be a regulator of inflammation, we have a potential relationship between the inflammatory process and B cell function. In addition, we have demonstrated that tumor necrosis factor alpha (TNF- α) enhances B cell proliferation and differentiation to Ig secretion. We are currently employing radiolabeled TNF- α to delineate the expression of TNF- α receptors on normal and transformed human B cells.

We have examined the role of complement components in B cell physiology and have demonstrated that C1q enhances the production of IgM in normal human B cells. In addition, we have demonstrated antigenic cross-reactivity between HMW-BCGF and the Bb fragment of Factor B. Interestingly, Bb was shown to enhance the proliferation of activated B cells while Ba had a potent suppressive effect.

Over the past year, we have continued our studies on the role of fibronectin and other matrix proteins in B cell physiology, especially the production of BCGF. We have shown that cell lines capable of producing BCGF have receptors for fibronectin and that interaction of fibronectin with that receptor results in BCGF production. Given the fact that fibronectin is a structural protein present in lymph nodes and has been shown to activate monocytes, it may also play a physiologic role in the regulation of B cell function.

We have continued our studies on the effects of arachadonic acid metabolites on human B cell function. We have previously demonstrated that leukotriene C4 (LTC4) was the suppressive factor in the supernatant of a T-T hybridoma which we had formerly shown suppressed Ig production. Over the past

year, we have demonstrated that LTC₄ binds preferentially to T cells rather than to B cells, and in fact suppressed T₄ cell proliferation while enhancing T₈ cell proliferation. These data suggest that the predominant effect of LTC₄ in suppressing B cell differentiation is mediated through T cells.

We had previously described a T₈⁺ T cell clone (YD2) which is a potent direct suppressor of B cell differentiation. We have recently demonstrated by reverse phase chromatography that the suppressive effect of the supernatant of YD2 was present only in the fractions which contained PGE₂. We further demonstrated that PGE₂ itself was a potent suppressor of B cell differentiation while not affecting B cell proliferation. Regulation of B cell Ig production by prostaglandins secreted by T cells has important implications in the relationship between the inflammatory response and B cell physiology. (Ambrus, Kehr, Goldstein, Bowen, T. Nakagawa, N. Nakagawa, Fauci, LIR/NIAID; Greene, MB/NCI; Lê thi Bich-Thuy, LB/NCI; Sporn, LCP/NCI; Brown, Washington University; Lewis, Austen, Harvard; Tenner, American Red Cross).

Pharmacologic Modulation of the Human Immune Response

Over the past 15 years, the LIR has been engaged extensively in the study of the effects of corticosteroids (CS) on the human immune response. We have examined the differential effects of in vitro CS on various stages in the human B cell cycle. A gradation of effect was noted on the discrete phases of B cell activation, proliferation, and differentiation in which the earliest activation steps in the cycle were most sensitive to the suppressive effects of CS, whereas the later events such as the proliferative response to BCGF are less sensitive. Over the past year, we have demonstrated that B cell proliferation induced directly by the calcium ionophore A23187 is not suppressed by CS. We have also shown that an early step in sIg-mediated signal transmission, sIg crosslinkage, and attachment to the cytoskeleton are not blocked by CS. These findings suggest that CS preferentially blocks the protein kinase-dependent signal transmission in G₀ phase B cells.

We had previously reported that cyclosporin A (CsA) selectively suppressed an early step in human B cell activation and had little inhibitory effect on the subsequent factor dependent proliferation and differentiation. Over the past year, we demonstrated that CsA differentially affected B cells according to their cell density which reflects their state of activation. High density B cells which are the most susceptible to Epstein-Barr virus (EBV) transformation manifested enhanced proliferation and differentiation in response to CsA. This may have implications in understanding the mechanisms for the development of malignancies in CsA-treated transplantation patients.

Finally, we have continued our studies on the effects of certain neuropeptides such as β endorphin on the cytolytic activity of large granular lymphocytes (LGL) and lymphokine activated killer (LAK) cells. β endorphin augmented the lytic activity of LGLs against K562 target cells but did not enhance LAK activity when present during the induction of LAK cells with IL-2. (Bowen, Kehr, Rook, T. Nakagawa, N. Nakagawa, Fauci, LIR/NIAID)

Clinical Correlates of Studies of Human B Cell Function

Extrapolating from our studies on the receptor for BCGF, we have begun to evaluate the BCGF receptor in patients with common variable hypogammaglobulinemia (CVH). While these patients respond poorly to HMW-BCGF

(but normally to LMW-BCGF), preliminary data suggest that they constitutively express BA5 protein (HMW-BCGF receptor) at a very high level. This suggests that either the BA5 protein is abnormal or signal transduction after interaction of HMW-BCGF with BA5 is abnormal. It is also possible that internalization of the BA5-HMW-BCGF complex is necessary for activity and this is deficient in these patients.

Finally, we have made T-T hybridomas from T cells of patients with systemic lupus erythematosus (SLE) and have studied in detail a factor produced by hybridomas from a patient whose disease flares correlated with increased IgG levels. This factor was shown to enhance IgG and suppress IgM production by normal B cells or EBV-transformed B cell lines. This study has relevance for understanding not only the enhanced Ig production in SLE but also the normal regulation of Ig production. (Ambrus, Young, Fauci, LIR/NIAID; Buckley, Duke).

Epidemiologic, Virologic, Molecular Biologic, Immunologic, Clinical, and Therapeutic Studies in the Acquired Immunodeficiency Syndrome (AIDS)

Over the past 4½ years, the LIR has been actively engaged in the study of multiple facets of AIDS, particularly the delineation of the immunopathogenesis of the syndrome and the design and implementation of therapeutic studies in patients with the disease. Over the past year, we have extended and intensified our studies to include the virology and molecular biology of the AIDS virus as well as certain aspects of the epidemiology of the disease.

In an ongoing study of over 500 employees of the NIH Clinical Center who have been followed with regard to antibody status for the AIDS virus, only 3 individuals were found to be antibody positive and each of these had one of the established risk factors for AIDS. The remainder of the hospital personnel involved in the care of AIDS patients or in the processing of AIDS specimens were seronegative, including 50 individuals who had needle-stick or mucosal splash exposure to AIDS virus-contaminated materials. These studies represent an important confirmation of the working hypothesis that the AIDS virus is not transmitted by casual contact and that the frequency of transmissibility from patient to hospital worker must be extraordinarily rare even with direct inoculation of potentially contaminated material.

In collaboration with the Syntex Corporation, a variety of DNA clones of the AIDS virus have been expressed in prokaryotic systems. Two of the proteins coded for by these clones have been found to reliably represent antigenic structures in the gag and envelope regions of the AIDS virus. Using these recombinant proteins, isotype-specific enzyme-linked immunosorbent assay (ELISA) systems have been established and it has been demonstrated that IgM antibody responses develop to gag and envelope proteins for a very brief period of time following infection and then disappear. Of great interest is the finding that seropositive individuals who develop strong antibody responses to gag proteins have a lower conversion rate to AIDS than do those who do not develop antibody to gag or who lose their response to gag. Larger numbers of patients will be studied in an attempt to fully confirm this observation which would have important implications in vaccine development.

We have previously developed a CD4+ T cell line (A3.01) which is easily infectable with and killed by the AIDS virus. Over the past year, we have demonstrated that following infection with the AIDS virus a CD4- line survives the infection and latently harbors the virus. This survivor cell line does not

actively produce infectious virus, but can be induced to do so. This cell line has important implications in understanding the mechanisms of viral latency as well as the factors involved in converting a latent infection to an actively productive infection. In addition, following induction of this latently infected line, we have cloned out several cell lines which appear to be genetic variants of the wild type AIDS virus. One such line, 8E5, was found to produce virus which did not express p66 (reverse transcriptase) and p34 (endonuclease) and was thus non-infectious. Variants of the AIDS virus such as these may be of value in enhancing our understanding of the biology of the AIDS virus as well as providing potential attenuated strains for consideration as vaccines.

In collaboration with NIAID's Laboratory of Molecular Microbiology, an infectious clone of the AIDS virus has been developed and a variety of non-lymphoid cells were transfected with the virus including a neuroblastoma line, a rhabdomyosarcoma line, and 3 colon cell lines. These studies have potentially important implications for understanding the scope of pathophysiological processes associated with AIDS virus infection.

We have adapted the technique of *in situ* hybridization using nucleic acid probes and have combined these with immunohistochemical staining to examine the expression of AIDS retroviral RNA sequences in tissue specimens from AIDS patients and in cell lines used as *in vitro* models of infection. Using this approach, we have demonstrated that the cell type in the human brain which is infected with the AIDS virus is a cell of histiocytic (monocyte/macrophage) origin. These cells form multinucleated giant cells in the brains of AIDS patients and have been shown by *in situ* hybridization to contain viral nucleic acid.

We have previously established the precise nature of the immunologic defect in AIDS. Over the past year, we have extended and expanded these studies. We have demonstrated that the subset of CD4⁺ cells which is affected early in the course of infection is the antigen-responsive T4 cell. We have also demonstrated that the peripheral blood monocyte in AIDS is defective in its function as an antigen-presenting cell. In addition, we are currently investigating the immunosuppressive properties which the virus and its components have directly on normal, non-infected T4 cells. In most recent studies using restriction map analysis of T cell alpha and beta genes, we have demonstrated that T cells in AIDS patients manifest a normal pattern of immunogenetic phenotypes but that a population of cells exist with incompletely rearranged genes suggesting that the depletion of T4 cells associated with infection with the AIDS virus may leave behind a population of immature T4 cells.

We had previously demonstrated that B cells from AIDS patients were polyclonally activated and deficient in their ability to respond to a *de novo* antigenic stimulus. We have recently demonstrated that the AIDS virus itself is a potent polyclonal B cell activator. Studies are currently underway employing synthetic peptides and recombinant proteins of the AIDS virus in an attempt to characterize the viral components giving rise to B cell activation and to identify a receptor for the virus on B cells.

We have demonstrated defects in the effector cells of antibody-dependent cellular cytotoxicity (ADCC) using chicken red blood cell targets. By employing labeled red blood cell clearance studies, we have further

demonstrated defects in both splenic and hepatic phagocytes in AIDS patients, particularly those with advanced disease.

In addition to studies on the immunopathogenesis of AIDS, we have initiated a series of studies on the host's response to infection with the AIDS virus. We have recently developed systems for measuring ADCC and HLA-restricted cytotoxic T cell responses against AIDS virus-infected cells. Sera from patients with AIDS generally mediated less ADCC than sera from healthy seropositive individuals, and enhanced ADCC seemed to correlate with the presence of antibodies to p24 (gag) as determined by Western blot analysis. These studies have important implications in vaccine development.

The LIR is heavily committed to developing and testing effective therapies for patients with AIDS, both with regard to the opportunistic infections and neoplasms (Kaposi's sarcoma) and the AIDS virus itself. We have participated in a study which demonstrated the value of dihydroxypropoxymethylguanine (DHPG) in the treatment of cytomegalovirus (CMV) syndromes in AIDS patients. We are currently involved in a study of the use of trimetrexate (a dihydrofolate reductase inhibitor) in the treatment of pneumocystosis and toxoplasmosis.

A variety of anti-retroviral agents have been tested and are being tested in the LIR. These include suramin, HPA-23, foscarnet, azidothymidine, and alpha interferon. In our open trial of alpha interferon in patients with Kaposi's sarcoma and AIDS, the drug appeared to be capable of inhibiting the virus (decreased ability to isolate the virus from treated patients), especially in those patients who experienced an anti-tumor effect.

The clinical trial using azidothymidine is just underway and so it is too early to comment on results. However, important information was gathered with our suramin trial. The drug was at first, based on a phase I trial in a limited number of patients, felt to show promise in the treatment of AIDS. However, our extended trial demonstrated that the drug was not effective as an antiviral agent, manifested considerable toxicity, and even accelerated the disease process in some patients. Suramin will no longer be used in clinical trials as a single agent in AIDS.

We undertook a clinical trial using recombinant interleukin (IL)-2 and demonstrated that this agent does hold promise as an immunomodulator in AIDS. Patients receiving IL-2 experienced enhancement of spontaneous lymphocyte proliferation, elevation in total lymphocyte counts, and a decline in the degree of polyclonal B cell activation. In addition to these findings of immunologic enhancement (which unfortunately were transient), both gross and histologic evidence of tumor regression were noted. We plan to use IL-2 in combination with an anti-retroviral agent once a safe and effective agent is identified. Our studies using gamma interferon have led us to conclude that as a single agent, it had little or no role in the treatment of AIDS both because of its toxicity and lack of demonstrable efficacy.

The LIR was one of the first laboratories to investigate the role of cellular reconstitution of the immune response in AIDS employing identical twin bone marrow transplantation and syngeneic lymphocyte transfusions. Over the past year, we have performed bone marrow transplants between 3 sets of identical twins, one of the pair with AIDS and the other well and seronegative. We combined these transplants with repetitive syngeneic lymphocyte transfusions and anti-retroviral therapy with suramin (prior to our demonstration of the

toxicity and lack of efficacy of suramin). One of the 3 transplant recipients has shown a significant and sustained (11 months as of 7/86) improvement in immunologic function following the procedures. There has been an increase in the number of T4 cells to normal, an appearance of delayed cutaneous hypersensitivity, and in vitro blastogenic response to soluble antigen. This latter observation constitutes correction of the earliest and perhaps the most specific immunologic defect in AIDS patients. Furthermore, this patient is doing very well clinically and is working full time. These studies establish the feasibility of reconstituting the defective immune response of an AIDS patient and is the first example of a sustained reversal of the immunologic defect in AIDS. Studies of immunologic reconstitution using bone marrow transplantation combined with anti-retroviral agents (if safe and effective agents become available) will be actively pursued in the LIR. (Lane, Folks, Margolick, Koenig, Rook, Schnittman, Quinn, Fauci, LIR/LIAID; Martin, Rabson, Benn, Gendelman, LMM/NIAID; Masur, Henderson, Alter, CC; Broder, Gelmann, COP/NCI).

International Studies of AIDS

In addition to our basic and clinical studies on AIDS, the LIR has been involved in studying the international aspects of the syndrome. AIDS has become a global pandemic, with cases reported in over 100 countries throughout the world. An intensive research effort has been undertaken in several countries, including the Caribbean, Africa, and India, to study the unique epidemiologic, virologic, clinical, and immunologic features of AIDS in these areas. In Kinshasa, Zaire, we have identified over 2,000 cases with a male-to-female ratio of 1:1. The disease is predominantly transmitted heterosexually based on a 72% transmission rate among spouses of AIDS patients, high seroprevalence rates among female prostitutes and sexually transmitted disease clinic populations. Other epidemiologic studies have demonstrated a 10% infection rate among hospitalized children and a 8% infection rate among blood bank donors in Africa. Case-control studies have confirmed needles, blood transfusions, and vertical transmission from mother to infant. HTLV-III/LAV has been isolated from selected African AIDS patients and genomic studies have demonstrated marked heterogeneity of African isolates compared to North American and European isolates. In clinical studies, a diarrheal-wasting syndrome was frequently observed, and 30% of hospitalized acute tuberculosis cases had evidence of infection with HTLV-III/LAV. Immunologic studies have also confirmed a marked elevation of activated CD4 lymphocytes suggesting that exposure to a wide variety of viral and parasitic infections may result in increased susceptibility to HTLV-III/LAV infection. Further studies will examine the natural history of HTLV-III/LAV infection in patients from developing countries with particular emphasis on perinatal transmission, safety, and efficacy of immunization programs in HTLV-III/LAV infected children and genomic changes in viral isolates. (Quinn, Lane, Folks, Francis, Lightfoote, Fauci, LIR/NIAID; Martin, Benn, LMM/NIAID; Mann, Curran, CDC; Piot, Colebunders, Institute of Tropical Medicine, Antwerp, Belgium; Holmes, Kreiss, University of Washington; Gerin, Wong, Fernic, Georgetown University)

Clinical, Immunopathogenic, and Therapeutic Studies in the Spectrum of Vasculitis

The LIR is currently studying prospectively the largest group of patients with the vasculitic syndromes in the world. On the basis of clinical, pathophysiologic, immunopathogenic, and therapeutic results obtained over the

past 18 years, we have designed a revised categorization scheme for the vasculitides which has now reached worldwide acceptance. In addition, we have described a new vasculitic syndrome which we have termed the polyangiitis overlap syndrome. We have developed and instituted aggressive chemotherapeutic regimens consisting of chronically administered cyclophosphamide together with alternate-day corticosteroids in several, formerly universally fatal diseases such as Wegener's granulomatosis. In this regard, we are now following over 140 patients with Wegener's granulomatosis in which we demonstrated a 93% remission and cure rate. We have now applied these approaches with remarkable success to other vasculitic syndromes such as systemic vasculitis of the polyarteritis nodosa group, isolated central nervous system vasculitis, Takayasu's arteritis, the acute vasculitis of Sjögren's syndrome, and lymphomatoid granulomatosis. LIR-designed therapeutic protocols for the vasculitic syndromes are now used worldwide. The patient populations studied in the vasculitis protocol have been utilized to precisely delineate aberrancies of lymphocyte activation and immunoregulation seen in these diseases. In addition, the precise effects of various therapeutic regimens, particularly corticosteroids and cytotoxic agents, on human lymphoid cells have been described. In this regard, we demonstrated the exquisite and selective sensitivity of certain phases of the B cell cycle to cyclophosphamide therapy, an observation which might help explain its efficacy in certain diseases characterized by hyperreactivity of B cell function. (Fauci, Leavitt, Lane, Rook, LIR/NIAID; Parrillo, CCM/CC; Cupps, Georgetown University)

Studies in Other Immune-Mediated Diseases

In 1984 we identified a phagocytosis-inducing factor derived from the lymphocytes of patients with erythrophagocytosis syndromes and angiocentric lymphoproliferative diseases as well as normals. Over the past year, we have biochemically characterized the factor and have identified the T4 cell as its cell of origin. We are currently in the process of purifying this factor with the plan to clone the genes for this novel lymphokine.

We have produced and characterized thyroid-derived T cell lines in autoimmune thyroid diseases (Graves' disease and Hashimoto's thyroiditis) and have delineated the heterogeneity of abnormalities of B cell function in these diseases. In addition, we have demonstrated the induction by interferon- γ of HLA-DR antigens on thyroid follicular cells. These studies shed new insight into the role of viral infection-induced interferon production in the pathogenesis of autoimmune thyroid disease.

We have continued our clinical and pathogenic studies on the idiopathic hypereosinophilic syndrome and have further characterized the specific components of the human eosinophil which play major roles in the pathogenesis of the syndrome. Over the past year, we have demonstrated that T cell clones from patients with eosinophilia and hyper-IgE secrete an antigen-specific factor which induced IgE isotype-specific Ig secretion from B cells in vitro. Finally, we are continuing our studies on the natural history, immunopathogenesis, and therapy of idiopathic dilated cardiomyopathy. The therapeutic trial should be completed in 1987, and preliminary results indicate that corticosteroids are effective in improving the underlying disease process. (Margolick, Volkman, Fauci, LIR/NIAID; Nutman, LPD/NIAID; Parrillo, CCM/CC; Jaffe, LP/NCI; Gleich, Mayo Clinic)

The Molecular Biologic Approach to the Human Immune System

The LIR has been actively pursuing studies on the activation and regulation of the human immune response at the molecular level. We have characterized nuclear molecular events during the activation of human T lymphocytes. The c-myc oncogene, the IL-2 growth factor, and the IL-2 receptor genes are known to be induced early during the activation of T cells. Chromatin structure analyses and some DNA-mediated transfection experiments have allowed us to identify several regulatory elements of these genes. In the case of the IL-2 gene, we have identified an element likely to be involved in the transmission of the extracellular activation signal. Along the same lines, a model system has been developed to study the effect of differentiation at the nuclear level. The promyelocytic leukemia cell line HL60 can be differentiated terminally in vitro. Concomitantly, the expression of the c-myc oncogene is downregulated. We have identified two distinct mechanisms to transcriptionally downmodulate c-myc, an early and a late-acting one; the latter one is associated with dramatic chromatin changes of the c-myc gene. We have also initiated more broadly based studies directed at the activation process of T cells. cDNA libraries have been made from activated peripheral blood T cells and have been used to identify genes which are specifically and immediately induced upon mitogenic stimulation. Several of these genes appear to be regulated in a manner analogous to that of the c-fos oncogene. These libraries are also utilized to identify genes coding for lymphokines which are elaborated by these T cells after stimulation. To clone the genes for the HMW-BCGF, we have also made libraries from a B cell tumor which expresses BCGF constitutively. Furthermore, we have initiated the cloning of the genes of the recently identified receptor for this growth factor by creating a cDNA library from an EBV-transformed B cell line expressing a relatively high number of these receptors. Lastly, we have used the purified BCGF to study its molecular effects on B cells. Specifically, we have determined that the c-myc gene is induced rapidly even on small B cells, which require a signal in addition to BCGF for proliferation. (Siebenlist, Bressler, Brunnvand, Zipfel, Ambrus, Fauci, LIR/NIAID; Kelly, IB/NCI; Greene, Leonard, MB/NCI; Holbrook, PD/NCI; Crabtree, Stanford University)

Studies of Chlamydia Trachomatis Infection

Chlamydia trachomatis is the most common sexually transmitted bacterial pathogen in the United States with 10 million cases annually. Studies have been carried out to further define the clinical spectrum of chlamydial infection, to develop rapid diagnostic assays, and to examine the pathogenesis of chlamydial infections in experimental animal models. In a study of 542 pregnant women, Chlamydia was present in 16% and was significantly associated with the clinical findings of cervicitis, preterm delivery, low birth weight infants, and conjunctivitis. In a subsequent study of 505 pregnant women presenting for induced abortions, C. trachomatis, present in 17% of patients, was also significantly associated with the development of postabortal endometritis. Newly developed diagnostic assays, such as immunofluorescent staining with monoclonal antibodies and in situ DNA hybridization were shown to have a relatively high sensitivity and specificity for the detection of Chlamydia. Development of a primate model of rectal C. trachomatis lymphogranuloma venereum (LGV) infection has aided in the study of the mucosal immune response to infection. Following rectal infection, there was reversal in the systemic T-cell lymphocyte populations and T-cell antigen-specific responses to LGV were demonstrated in the peripheral blood and mesenteric lymph

nodes but not in the lamina propria. This failure to respond to C. trachomatis antigen within the mucosa correlated with persistence of C. trachomatis in tissue histiocytes as determined by immunofluorescence and in situ DNA hybridization. In summary, the above studies demonstrate the diverse clinical spectrum of C. trachomatis infection, and provide methods for the rapid screening of Chlamydia and for studying the mucosal immune response to C. trachomatis. (Quinn, LIR/NIAID; James, Strober, LCI/NIAID)

Future Plans and Objectives

Over the past year, the LIR has made a number of significant advances in the study of the factors and receptors for the factors involved in the activation, proliferation, and differentiation of human B lymphocytes. We have purified the HMW-BCGF and partially sequenced it. Studies will be actively pursued towards cloning the genes for this factor. Over the past year, we identified the cellular protein BA5 which serves as the receptor for HMW-BCGF. Over the coming year, studies will be directed at more precise characterization of this receptor as well as cloning the genes for this receptor.

We will continue our studies on factors such as our BCAF which directly activate resting B cells. We will attempt to identify and isolate the cellular receptor for this direct B cell activating factor. In addition, we will aim studies at purifying and sequencing the factor in preparation for cloning its genes.

We will continue and expand our studies on the B cell clones which selectively produce and respond to BCGF in order to more precisely delineate the requirements for production and/or response to BCGFs.

We have previously developed and described a T cell clone (YA2) which selectively secretes BCDF, but not BCGF. Over the past year, we have purified this factor and have demonstrated its selective binding to BCDF-responsive cell lines. Over the coming year, we will further purify and partially sequence this factor. Attempts will be made to identify and isolate the cellular factor for BCDF and to clone the genes for both the factor itself and its receptor.

We will continue our studies on the role of IL-2 and IL-2 receptors on B cell proliferation and differentiation. We will attempt to dissect out more precisely the similarities and differences in the B cell response to IL-2 versus those factors such as BCGF and BCDF which are apparently more specific for B cells.

Over the past year, we have studied the effects of a variety of factors on B cell function which are not classically described B cell trophic factors. These have included TGF- β , TNF- α , and Clq. We will continue to pursue such studies. Furthermore, we will continue our studies on the effects of arachadonic acid metabolites such as LTC4 and PGE2 on human B cell function.

We will continue to pursue studies aimed at delineating precisely the mechanisms of action of corticosteroids on human immune function, particularly the various phases of the B cell cycle.

Our observation over the past year that lymphocytes from patients with CVH respond poorly to HMW-BCGF, but nonetheless constitutively express receptors for the factor, will be actively pursued. We will investigate whether the receptor itself is abnormal or whether signal transduction after interaction between factor and receptor for factor is abnormal.

We will continue and expand our studies on the molecular events which occur during the activation process involving human T cells. Particularly, we will pursue our chromatin structural analytical studies aimed at identifying the regulatory elements of the c-myc oncogene, the IL-2, and IL-2 receptor genes.

We will continue and intensify our studies on the epidemiologic, virologic, molecular biologic, immunologic, clinical, and therapeutic studies in AIDS. In particular, we will pursue our studies delineating the precise nature of the immunopathogenic mechanisms operable with HTLV-III/LAV infection. In addition, we will continue to delineate the scope of immune responses to the AIDS retrovirus. We will attempt to isolate the receptor on human B cells for the AIDS virus and will pursue further the role of the monocyte in viral pathogenesis. We will continue our studies on the mechanisms of conversion from viral latency to productive infection. Our studies on the relationship between the type of antibody response, i.e., anti-gag versus anti-env, and the clinical course of patients will be actively pursued. We will also continue studies on genetic variants of the wild type AIDS virus in order to investigate attenuated strains and their potential role in vaccine development.

A considerable effort will be devoted to our studies on anti-retroviral and immunomodulator clinical trials. Of particular note are our studies on bone marrow transplantation and lymphocyte transfusions between identical twin pairs. Our early encouraging results in this area serve as a basis for vigorous pursuit of this approach to immunologic reconstitution in AIDS patients.

Our international studies on AIDS will continue both in Africa and the Caribbean and will be extended to India and certain countries in South America.

Our studies on the clinical, immunopathogenic, and therapeutic aspects of the spectrum of vasculitic syndromes will be actively pursued. They have resulted in extraordinary contributions in the past, particularly with regard to the design and implementation of therapeutic regimens in these diseases.

Studies on the clinical, epidemiologic, and immunopathogenic aspects of chlamydial infections will continue in collaboration with the Johns Hopkins University Medical Center.

Administrative, Organization, and Other Changes

The Laboratory of Immunoregulation (LIR) was established in late 1980 and is now 6 years old. The theme of the LIR is the study of the mechanisms of activation and immunoregulation of human immune responses in normal individuals and in a variety of disease states characterized by abnormalities of immune function. In addition to basic research ranging from cellular physiology to molecular biology, the LIR continues to conduct a major portion of the clinical studies which are carried out in the NIAID Intramural Research Program within the NIH Clinical Center.

Over the past year, there were a number of administrative changes which have had an impact on the LIR. Dr. Anthony S. Fauci, who is Director of the NIAID in addition to the Chief of the LIR, was appointed as the Coordinator of AIDS Research for the entire NIH. Dr. H. Clifford Lane assumed major responsibility for conducting the Clinical Studies on AIDS in the LIR. Dr. Lane continues his role as the Deputy Clinical Director of the NIAID as he coordinates this wide range of clinical studies. The Clinical Staff under Dr. Lane has expanded with the inclusion of nurses and other support personnel such as Stephanie Carlton and Betsey Herpin, who function within the LIR as study coordinators.

Dr. Randi Y. Leavitt has assumed increasing responsibilities in assisting Dr. Fauci in the conduction of Clinical Research in the LIR. She has been converted to the Commissioned Officer Corps of the USPHS and will continue as a Senior Investigator. Dr. Julian L. Ambrus, Jr., was also converted to the Commissioned Corps and has been made a Senior Investigator in the LIR. Dr. Harris Goldstein will continue on for a fourth year as a Senior Staff Fellow. Drs. K. Randall Young, Jean C. Evans, and Steven Schnittman joined the LIR as first year Medical Staff Fellows. Dr. Ulrich Siebenlist, who heads the molecular biology component of the LIR, was converted from a Visiting Fellow to a Visiting Scientist. Additions to his group have included Dr. Albrecht-Georg Schmidt, a Guest Researcher. Drs. Scott Koenig and Peter Bressler have entered their second year of medical staff fellowship in 1985-1986. Dr. Jose R. Balea has also joined the laboratory as a Guest Researcher.

Dr. Shohken Tomita has transferred from the LIR to a Visiting Fellowship in the Surgery Branch of the NCI. Drs. Toshimasa Nakagawa and Naoko Nakagawa have entered their third year as Visiting Fellows. Drs. Debra L. Bowen and Alain H. Rook left the LIR in July 1986. Also leaving the laboratory were biologists Ms. Julia H. Grove, Ms. Sandra E. Higgins, Ms. Esther Racoosin, and Ms. Cynthia H. Jurgensen. Mr. Patrick J. McFarland, a biologist, joined the LIR to work with Dr. Ambrus.

Ms. Doris Light has been appointed as Secretary to the Chief, LIR, replacing Ms. Joan Eccard, and Ms. Mary Rust was appointed as Editorial Assistant replacing Ms. Dee Goodrich.

The laboratory space remains consolidated in the B wing of the 11th floor of the Clinical Center.

Honors, Awards, and Scientific Recognition

Over the past year, members of the LIR, particularly in the persons of Drs. Anthony S. Fauci, H. Clifford Lane, and Thomas C. Quinn, have received a number of awards and honors.

Dr. Fauci continues to serve on a number of committees of scientific and administrative importance. Over the past year, he was appointed a Deputy Ethics Counsellor of the Department of Health and Human Services (DHHS). He was appointed as the Coordinator of AIDS Research for the NIH. He was appointed a member of the DHHS Committee to Coordinate Environmental and Related Programs; a member of the Scientific Organizing Committee of the Joint Meeting of the Italian Society of Immunology and Immunopathology - National Institutes of Health on Human Lymphocyte Activation; a member of the United States Delegation to the United States - Japan Cooperative Medical Science Committee; a member of the Scientific Seminar Subcommittee of the National Institutes of Health Centennial Program; a member of the Scientific Organizing Committee of the International Conference on Lymphocyte Activation and Immune Regulation; a member of the Planning Committee of the Society for Clinical Immunology; a member of the Advisory Committee for the International Conference on AIDS. He enters the third year of his term as a member of the Board of Directors of the American Board of Allergy and Immunology. Dr. Lane is Chairman of the Medical Arts and Photography Branch Advisory Committee and is a member of the AIDS Drug Selection Committee and the Normal Volunteer Advisory Committee at the NIH. He also is a member of the committee for the review of immunologic studies in space for the Federation of American Societies for Experimental Biology. Dr. Quinn was appointed to the Advisory Committees on AIDS to the Pan American Health Organization (PAHO) and the Institute of Medicine of the National Academy of Sciences and is a World Health Organization/PAHO consultant to the Ministry of Health of Cuba, the Ministry of Health of Zaire, the Ministry of Health of Kenya, the Ministry of Health of India, and the Ministry of Health of Trinidad. Dr. Quinn also serves as an advisor to the AIDS Committee of Puerto Rico.

Dr. Fauci serves on a number of editorial boards of journals concerned with the areas of immunology, allergy, and infectious diseases. He continues his role as Associate Editor in charge of Allergy and Immunology of the American Journal of Medicine. He maintains his position on the Editorial Boards of Clinics in Immunology and Allergy, The Annals of Allergy, The Journal of Immunopharmacology, EOS, Clinical and Experimental Rheumatology, La Ricerca, Clinical Immunology and Immunopathology, Immunologia Clinica e Sperimentale, Physician's Journal Update, Immunopharmacology, the Journal of Molecular and Cellular Immunology, and Cellular Immunology. In addition, he continues his appointment as an Advisory Editor for North America for the journal Thymus and for the Journal of Clinical Immunology. Over the past year, he was appointed a member of the Advisory Editorial Board of the Clinical Immunology Newsletter. He continues to co-edit with Dr. John I. Gallin the book series ADVANCES IN HOST DEFENSE MECHANISMS. In addition, he co-edits the textbooks CURRENT THERAPY IN ALLERGY, IMMUNOLOGY, AND RHEUMATOLOGY and CURRENT THERAPY IN INTERNAL MEDICINE. Of particular note is the fact that he is an editor of the internationally renowned textbook of medicine, HARRISON'S PRINCIPLES OF INTERNAL MEDICINE. Finally, Dr. Fauci has contributed a number of invited chapters covering a variety of subjects for most of the major textbooks of medicine as well as subspecialty textbooks in immunology, allergy, and infectious diseases. Dr. Quinn is on the editorial board of the journal

Sexually Transmitted Diseases. In addition, he is co-editor of two Symposia published in Reviews of Infectious Diseases entitled International Symposium on Measles Immunization and International Symposium on Poliomyelitis Immunization. Dr. Quinn is also co-editor of the volume entitled TOPICS IN ACQUIRED IMMUNODEFICIENCY SYNDROME (AIDS).

As part of the recognition for scientific accomplishments, clinical investigators may be asked to visit outside institutions and serve for periods of from 2 to 5 days as Visiting Professor within a given institution. In this regard, Dr. Fauci has served as Visiting Professor at major medical centers throughout the country over the years. During the past year, he was the Leo H. Crip, M.D. Visiting Professor of Medicine at the Montefiore Hospital-University of Pittsburgh School of Medicine. In addition, he was the Ninth Annual Golan Memorial Visiting Professor of the Department of Pediatrics of the North Shore University Hospital-Cornell Medical Center.

Dr. Fauci was asked to deliver several major or named lectureships during the year. He was an Invited Plenary Speaker at the Sixty-Third Annual Meeting of the American College Health Association, the Annual Meeting of the American Society for Virology, and the Joint Meeting of the Italian Society of Immunology and Immunopathology - National Institutes of Health. He was a Symposium Speaker at the 1985 Annual Meeting of the American Association of the Advancement of Science and the Cold Spring Harbor Symposium on Modern Approaches to Vaccines, where he was also a session chairman. Dr. Fauci delivered the John H. Erskine Lecture at St. Jude's Children's Research Hospital, the Macombre Lecture at Harvard Medical School, the 1985 Dwight Griswald Memorial Lectureship at Hartford Hospital in Connecticut, and the Kroc Foundation Distinguished Lectureship of the Western Association of Physicians. He was an Invited Plenary Speaker at the Roche-UCLA Symposium on Viruses and Human Cancer, the International Conference on Lymphocyte Activation and Immune Regulation, and the 139th Annual Meeting of the American Psychiatric Association. In addition, he was an Invited Plenary Lecturer at the Annual Meeting of the American Academy of Allergy and Immunology and the National Council for International Health 13th Annual Conference. He was an Invited Symposium Speaker at Seminars in Advanced Rheumatology, the 123rd Annual Meeting of the National Academy of Sciences, and the 6th International Congress of Immunology. Dr. Fauci was an Invited Plenary Speaker at the International Conference on AIDS, the 6th International Congress on Sexually Transmitted Diseases, and the First International Union of Immunological Societies Conference on Clinical Immunology. Dr. Fauci participated in the Meet the Professor program at the Annual Meeting of the American College of Physicians and was an Invited Panel Discussant at the Annual Meeting of the American College of Physicians. He delivered The Leo H. Crip, M.D. Lectureship at Montefiore Hospital, University of Pittsburgh School of Medicine, the 20th Annual Knowles Lectureship of the Northern California Chapter of the Arthritis Foundation, The Donald Berkowitz Memorial Lectureship of the Medical College of Pennsylvania, the Fourth Annual Clemens von Pirquet Lectureship of Georgetown University Medical Center, and the Ninth Annual Golan Memorial Lectureship of the Department of Pediatrics, North Shore University Hospital. Dr. Fauci also delivered the prestigious 10th Annual Meadow Brook Lectureship. Dr. Lane was an invited lecturer at the national meeting of the American College of Clinical Pharmacology, the National Medical Association, the American Association of Blood Banks, the American Academy of Dermatology, and the Second International Conference on AIDS. He was a workshop chairman at the U.S. Public Health Service Professional Association meeting. Dr. Quinn delivered invited

lectureships at the annual meeting of SREPCIM, the American Society of Microbiology, the American College of Physicians, and the annual meeting of the Armed Forces Institute of Pathology. In addition, he was an invited lecturer at the First Conference on the Acquired Immunodeficiency Syndrome and Kaposi's Sarcoma and at the following institutions: Wayne State University School of Medicine, University of Indiana Medical Center, Harvard School of Public Health and Hygiene, Yale University School of Medicine, and University of Washington School of Medicine.

A 1985 Stanford University Arthritis Center Survey of the American Rheumatism Association membership ranked the work of Dr. Anthony S. Fauci on the treatment of polyarteritis nodosa and Wegener's granulomatosis as one of the most important advances in patient management in rheumatology over the past 20 years.

Finally, over the past year Dr. Fauci was honored with the prestigious Clemens von Pirquet Award of Georgetown University Medical Center. Dr. Quinn received the U.S. Public Health Service Commendation Award.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00210-06 LIR
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Immunoregulation of Human Lymphocyte Function in Normal and Disease States		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Anthony S. Fauci	Chief LIR, NIAID
Others:	John H. KehrI	Senior Investigator LIR, NIAID
	Julian L. Ambrus, Jr.	Senior Staff Fellow LIR, NIAID
	Harris Goldstein	Senior Staff Fellow LIR, NIAID
	Debra L. Bowen	Senior Staff Fellow LIR, NIAID
	Ulrich K. Siebenlist	Visiting Scientist LIR, NIAID
	K. Randall Young	Medical Staff Fellow LIR, NIAID
COOPERATING UNITS (if any) MB, NCI, W. Greene; LCP, NCI, M. Sporn; Washington Univ., E. Brown; Harvard Univ., R. Lewis, K.F., Austen; American Red Cross, A. Tenner; Duke Univ., R. Buckley; Univ. Minn., F. Uckun; Georgetown Univ., T. Cupps.		
LAB/BRANCH Laboratory of Immunoregulation		
SECTION		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS 3	PROFESSIONAL: 2	OTHER: 1
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The mechanisms of activation, proliferation and differentiation of human B cells were studied at the cellular and molecular levels. A T cell-derived B cell activating factor was identified which directly activated resting human B cells. A high molecular weight B cell growth factor (HMW-BCGF) which we had previously described was shown to induce c-myc expression in resting B cells in the absence of subsequent proliferation. The receptor for HMW-BCGF was identified and characterized on human B cells. This constitutes the first description of a B cell growth factor receptor. Separate clones derived from a B cell lymphoma were shown to selectively produce or respond to BCGF. The precise role of interleukin 2 (IL-2) and IL-2 receptors in B cell function were studied and the selective effect of IL-2 versus B cell differentiation factor on human B cells was demonstrated to depend on the state of maturation of the responding cell. The effects of complement components and fibronectin proliferation on the differentiation of human B cells were described. Metabolites of arachadonic acid such as leukotriene C4 and prostaglandin E2 were shown to have profound effects on B cell function indicating an important link between the inflammatory response and B cell function. Abnormalities of BCGF receptor formation were described in patients with common variable hypogammaglobulinemia constituting the first recognition of an abnormality at the level of interaction between BCGF and its receptor in a human disease. The pharmacologic modulation of the human immune response was studied, particularly with regard to the effects of corticosteroids, cyclosporin A, and certain neuropeptides on distinct phases of the human B cell cycle. </p>		

Others:	Jean Evans	Medical Staff Fellow	LIR, NIAID
	Toshimasa Nakagawa	Visiting Fellow	LIR, NIAID
	Naoko Nakagawa	Visiting Fellow	LIR, NIAID
	Shohken Tomita	Visiting Fellow	LIR, NIAID

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00212-06 LIR
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Study of the Immunopathogenic Features of Immune-Mediated Diseases		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Anthony S. Fauci	Chief LIR, NIAID
Others:	Joseph B. Margolick	Medical Staff Fellow LIR, NIAID
	H. Clifford Lane	Senior Investigator LIR, NIAID
	Julian L. Ambrus, Jr.	Senior Staff Fellow LIR, NIAID
	K. Randall Young	Medical Staff Fellow LIR, NIAID
	Anthony P. Weetman	Guest Researcher LIR, NIAID
	Alain H. Rook	Senior Investigator LIR, NIAID
COOPERATING UNITS (if any) LCI, NIAID, J. I. Gallin; CPD, CC, H. Gralnick; CCM, CC, J. E. Parrillo; LP, CC, E. Jaffe; Mayo Clinic, Rochester, MN, G. Gleich; University of Washington, Seattle, W. Henderson; NIADDK, B. Weintraub		
LAB/BRANCH Laboratory of Immunoregulation		
SECTION		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS 2	PROFESSIONAL: 2	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The <u>immunopathogenic mechanisms</u> of a number of <u>immune-mediated diseases</u> and/or diseases characterized by abnormalities of immune function were investigated. We identified and characterized a <u>phagocytosis-inducing factor (PIF)</u> derived from the T cells of patients with <u>granulomatous and erythrophagocytotic disorders</u>, including the <u>angioinvasive immunoproliferative diseases</u>. These studies have important implications in understanding the mechanisms of secondary immune-mediated disease associated with specific T cell derived factors. We produced and characterized thyroid-derived T cell lines in <u>autoimmune thyroid diseases</u> (Graves' disease and Hashimoto's thyroiditis) and delineated the heterogeneity of abnormalities in B cell function in these diseases. In addition, we demonstrated the induction by interferon-γ of HLA-DR antigens on thyroid follicular cells. These studies shed new insight into the role of viral infection-induced interferon production in the pathogenesis of autoimmune thyroid disease. We have extended our studies on the <u>idiopathic hypereosinophilic syndrome (HES)</u> and have demonstrated that T cell clones from patients with eosinophilia and hyper-IgE secrete an antigen-specific factor which induces IgE isotype specific Ig secretion from B cells in vitro. Finally, we have demonstrated that patients with active <u>systemic lupus erythematosus</u> have circulating T cells which selectively secrete an IgG-specific B cell differentiation factor. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00213-06 LIR
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Clinical, Immunopathogenic, and Therapeutic Studies in the Spectrum of Vasculitis		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Anthony S. Fauci	Chief LIR, NIAID
Others:	Randi Y. Leavitt H. Clifford Lane Alain H. Rook	Senior Investigator LIR, NIAID Senior Investigator LIR, NIAID Medical Officer LIR, NIAID
COOPERATING UNITS (if any) CCM, CC, J. E. Parrillo, J. Shelhamer; Georgetown University, T. R. Cupps		
LAB/BRANCH Laboratory of Immunoregulation		
SECTION		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	2	0
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) <p> Vasculitis is a pathophysiologic process which is involved in a spectrum of clinical syndromes. The underlying mechanism of this process is in most cases an aberrant immunologic response. The LIR is currently studying prospectively the largest group of patients with the <u>vasculitic syndromes in the world</u>. On the basis of <u>clinical, pathophysiologic, immunopathogenic and therapeutic results</u> obtained over the past 18 years, we have designed a <u>revised categorization scheme</u> for the vasculitides which has now reached <u>worldwide acceptance</u>. In addition, we have described a new vasculitis syndrome which we have termed the <u>polyangiitis overlap syndrome</u>. We have developed and instituted aggressive chemotherapeutic regimens consisting of chronically administered cyclophosphamide together with alternate-day corticosteroids in several, <u>formerly universally fatal diseases such as Wegener's granulomatosis</u>. In this regard, we are now following over 140 patients with <u>Wegener's granulomatosis</u> in which we demonstrated a <u>93% remission and cure rate</u>. We have now applied these approaches with remarkable success to other of the vasculitic syndromes such as <u>systemic vasculitis of the polyarteritis nodosa group, isolated central nervous system vasculitis, Takayasu's arteritis, the acute vasculitis of Sjögren's syndrome, and lymphomatoid granulomatosis</u>. The patient populations studied in the vasculitis protocol have been utilized to precisely delineate aberrancies of <u>lymphocyte activation and immunoregulation</u> seen in these diseases. In addition, the precise effects of various <u>therapeutic regimens, particularly corticosteroids and cytotoxic agents, on human lymphoid cells</u> have been described. In this regard, we demonstrated the exquisite and selective sensitivity of certain phases of the B cell cycle to cyclophosphamide therapy, an observation which might help explain its efficacy in certain diseases characterized by hyperreactivity of B cell function. Furthermore we have delineated the spectrum of effects of corticosteroids on the various phases of the <u>human B cell cycle</u>. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00390-03 LIR																					
PERIOD COVERED October 1, 1985 to September 30, 1986																							
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Studies of the Acquired Immunodeficiency Syndrome																							
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: H. Clifford Lane</td> <td style="width: 33%;">Senior Investigator</td> <td style="width: 33%;">LIR, NIAID</td> </tr> <tr> <td>Others: Anthony S. Fauci</td> <td>Chief</td> <td>LIR, NIAID</td> </tr> <tr> <td>Thomas M. Folks</td> <td>Expert</td> <td>LIR, NIAID</td> </tr> <tr> <td>Joseph B. Margolick</td> <td>Medical Staff Fellow</td> <td>LIR, NIAID</td> </tr> <tr> <td>Scott Koenig</td> <td>Medical Staff Fellow</td> <td>LIR, NIAID</td> </tr> <tr> <td>Marilyn M. Lightfoote</td> <td>Staff Fellow</td> <td>LIR, NIAID</td> </tr> <tr> <td>Thomas C. Quinn</td> <td>Senior Investigator</td> <td>LIR, NIAID</td> </tr> </table>			PI: H. Clifford Lane	Senior Investigator	LIR, NIAID	Others: Anthony S. Fauci	Chief	LIR, NIAID	Thomas M. Folks	Expert	LIR, NIAID	Joseph B. Margolick	Medical Staff Fellow	LIR, NIAID	Scott Koenig	Medical Staff Fellow	LIR, NIAID	Marilyn M. Lightfoote	Staff Fellow	LIR, NIAID	Thomas C. Quinn	Senior Investigator	LIR, NIAID
PI: H. Clifford Lane	Senior Investigator	LIR, NIAID																					
Others: Anthony S. Fauci	Chief	LIR, NIAID																					
Thomas M. Folks	Expert	LIR, NIAID																					
Joseph B. Margolick	Medical Staff Fellow	LIR, NIAID																					
Scott Koenig	Medical Staff Fellow	LIR, NIAID																					
Marilyn M. Lightfoote	Staff Fellow	LIR, NIAID																					
Thomas C. Quinn	Senior Investigator	LIR, NIAID																					
COOPERATING UNITS (if any) CCM, CC, H. Masur, H. Alter, D. Henderson; COP, NCI, S. Broder, E. Gelmann; LMM, NIAID, M. Martin, A. Rabson, S. Benn, H. Gendelman, A. Adachi, LIG, NIAID, T. Kindt, M. Robinson, A. Lew.																							
LAB/BRANCH Laboratory of Immunoregulation																							
SECTION																							
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892																							
TOTAL MAN-YEARS 3	PROFESSIONAL: 2	OTHER: 1																					
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews																							
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> An intensive effort was directed at studying <u>epidemiologic, virologic, immunologic and clinical aspects of the acquired immunodeficiency syndrome (AIDS)</u>. An ongoing study of 500 hospital employees has demonstrated the low risk of infection with the AIDS virus among hospital personnel. ELISA techniques have been developed for measuring <u>isotype specific responses to proteins of the AIDS virus</u> and these studies have demonstrated a better prognosis for those infected individuals who mount strong antibody responses to <u>gag proteins</u>. A cell line constitutively producing non-infectious AIDS virus was characterized and found to lack two of the polymerase proteins. A variety of non-lymphoid cell lines were found capable of supporting replication of the AIDS virus including colon cells. Virus was detected in multinucleated giant cells of the brains of patients with <u>AIDS encephalopathy</u> constituting the first demonstration of the precise cell type in the brain infected with the AIDS virus. Monoclonal antibodies directed against the AIDS virus were generated. The nature of the loss of antigen specific reactivity of the immune systems of patients with AIDS was further characterized by demonstrating a normal family of T cell receptor gene phenotypes and the presence of intact T cell receptor proteins on the surface of T cells obtained from patients with AIDS. The AIDS virus was found to be a potent <u>polyclonal B cell activator</u>. The nature of the immune response to the AIDS virus was examined utilizing systems for measuring antibody dependent cytotoxicity and cytotoxic T cells. A series of clinical trials were conducted in an attempt to improve the clinical status of patients with AIDS. The drug <u>DHPG</u> was studied in cytomegalovirus disease and trimetrexate studied in patients with pneumocystosis or toxoplasmosis. Among the <u>anti-retroviral agents</u> tested were <u>suramin, HPA-23, foscarnet, and alpha interferon</u>. Among the <u>immunomodulatory approaches</u> taken were <u>interleukin-2 therapy and identical twin bone marrow transplantation</u> coupled with peripheral lymphocyte transfers. </p>																							

Others:	Steven Schnittman	Medical Staff Fellow	LIR, NIAID
	Margaret Megill	Nurse Practitioner	LIR, NIAID
	Betsey Herpin	Project Coordinator	LIR, NIAID
	Stephanie Carleton	Project Coordinator	LIR, NIAID
	Linda Thompson	Project Coordinator	LIR, NIAID

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00431-02 LIR
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) The Molecular Biologic Approach to the Immune System		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Ulrich K. Siebenlist	Visiting Scientist LIR, NIAID
Others:	Peter Bressler	Medical Staff Fellow LIR, NIAID
	Mark W. Brunvand	National Research Service Award Fellow LIR, NIAID
	Peter F. Zipfel	Guest Researcher LIR, NIAID
	Anthony S. Fauci	Chief LIR, NIAID
	Julian L. Ambrus, Jr.	Senior Staff Fellow LIR, NIAID
COOPERATING UNITS (if any) IB, NCI, K. Kelly; MB, NCI, W. Greene, W. Leonard; PD, NCI, N. Holbrook; Stanford University, J. Crabtree.		
LAB/BRANCH Laboratory of Immunoregulation		
SECTION		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS 3	PROFESSIONAL 2	OTHER 1
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> We have characterized nuclear <u>molecular</u> events during the activation of human T lymphocytes. The <u>c-myc oncogene</u>, the <u>interleukin-2 (IL-2)</u> growth factor and the <u>IL-2 receptor gene</u> are known to be induced early during the activation of T cells. <u>Chromatin structure</u> analyses and some DNA mediated <u>transfection</u> experiments have allowed us to identify several <u>regulatory</u> elements of these genes. In the case of the IL-2 gene, we have identified an element likely to be involved in the transmission of the extracellular activation signal. Along the same lines, a model system has been developed to study the effect of differentiation at the nuclear level. The promyelocytic leukemia cell line <u>HL60</u> can be differentiated terminally in vitro. Concomitantly, the expression of the c-myc oncogene is downregulated. We have identified two distinct mechanisms to transcriptionally downmodulate c-myc, an early and a late acting one; the latter one is associated with dramatic chromatin changes of the c-myc gene. We have initiated also more broadly based studies directed at the activation process of <u>T cells</u>. <u>cDNA</u> libraries have been made from activated peripheral blood T cells and have been used to identify genes which are specifically and immediately induced upon mitogenic stimulation. Several of these genes appear to be regulated in a manner analogous to that of the <u>c-fos oncogene</u>. These libraries are utilized also to identify genes coding for <u>lymphokines</u> which are elaborated by these T cells after stimulation. To clone the well-characterized B cell growth factor (<u>BCGF</u>), we have also made libraries from a B cell tumor which expresses BCGF constitutively. Furthermore, we have initiated the cloning of the recently identified <u>receptor</u> for this growth factor by creating a cDNA library from an Epstein-Barr virus transformed B cell line expressing a relatively high number of these receptors. Lastly, we have used the purified BCGF to study its molecular effects on B cells. Specifically, we have determined that the c-myc oncogene is induced rapidly even on small B cells, which require a signal in addition to BCGF for <u>proliferation</u>. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00361-04 LIR																		
PERIOD COVERED October 1, 1985 to September 30, 1986																				
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) International Studies of the Acquired Immunodeficiency Syndrome																				
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: Thomas C. Quinn</td> <td style="width: 33%;">Senior Investigator</td> <td style="width: 33%;">LIR, NIAID</td> </tr> <tr> <td>Others: Anthony S. Fauci</td> <td>Chief</td> <td>LIR, NIAID</td> </tr> <tr> <td>H. Clifford Lane</td> <td>Senior Investigator</td> <td>LIR, NIAID</td> </tr> <tr> <td>Thomas M. Folks</td> <td>Expert</td> <td>LIR, NIAID</td> </tr> <tr> <td>Henry L. Francis</td> <td>Expert</td> <td>LIR, NIAID</td> </tr> <tr> <td>Maryilyn M. Lightfoote</td> <td>Staff Fellow</td> <td>LIR, NIAID</td> </tr> </table>			PI: Thomas C. Quinn	Senior Investigator	LIR, NIAID	Others: Anthony S. Fauci	Chief	LIR, NIAID	H. Clifford Lane	Senior Investigator	LIR, NIAID	Thomas M. Folks	Expert	LIR, NIAID	Henry L. Francis	Expert	LIR, NIAID	Maryilyn M. Lightfoote	Staff Fellow	LIR, NIAID
PI: Thomas C. Quinn	Senior Investigator	LIR, NIAID																		
Others: Anthony S. Fauci	Chief	LIR, NIAID																		
H. Clifford Lane	Senior Investigator	LIR, NIAID																		
Thomas M. Folks	Expert	LIR, NIAID																		
Henry L. Francis	Expert	LIR, NIAID																		
Maryilyn M. Lightfoote	Staff Fellow	LIR, NIAID																		
COOPERATING UNITS (If any) MM, NIAID, M. Martin, S. Benn: CDC, J. Mann, J. Curran; Institute Tropical Medicine, Antwerp, Belgium, P. Piot, R. Colebunders; Univ. of Washington, K. Holmes, J. Kreiss; Georgetown Univ., J. Gerin, D. Wong, B. Fernic.																				
LAB/BRANCH Laboratory of Immunoregulation																				
SECTION NIAID, NIH, Bethesda, Maryland 20892																				
INSTITUTE AND LOCATION																				
TOTAL MAN-YEARS: 5	PROFESSIONAL: 3	OTHER: 0																		
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews																				
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The <u>acquired immunodeficiency syndrome (AIDS)</u> has become a <u>global pandemic</u>, with cases reported in over 100 countries throughout the world. An intensive research effort has been undertaken in several countries, including the Caribbean, Africa, and India to study the <u>unique epidemiologic, virologic, clinical and immunologic features of AIDS</u> in these areas. In Kinshasa, Zaire, we have identified over 2,000 cases with a male-to-female ratio of 1:1. The disease is predominantly transmitted heterosexually based on a 72% transmission rate among spouses of AIDS patients, <u>high seroprevalence rates among female prostitutes and STD clinic populations</u>. Other epidemiologic studies have demonstrated a 10% infection rate among hospitalized children and a 8% infection rate among blood bank donors in Africa. Case-control studies have confirmed needles, blood transfusions, and vertical transmission from mother to infant. HTLV-III/LAV has been isolated from selected African AIDS patients and genomic studies have demonstrated marked heterogeneity of African isolates compared to North American and European isolates. In clinical studies a <u>diarrheal-wasting syndrome</u> was frequently observed, and 30% of hospitalized acute tuberculosis cases had evidence of infection with HTLV-III/LAV. Immunologic studies have also confirmed a marked elevation of <u>activated CD4 lymphocytes</u> suggesting that exposure to a wide variety of viral and parasitic infection may result in increased <u>susceptibility</u> to HTLV-III/LAV infection. Further studies will examine the <u>natural history of HTLV-III/LAV infection in patients of developing countries with particular emphasis on perinatal transmission, safety and efficacy of immunization programs in HTLV-III/LAV infected children, and genomic changes in viral isolates.</u> </p>																				

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00358-04 LIR
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Immunopathogenesis of Chlamydia Trachomatis Infection.		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <div style="display: flex; justify-content: space-between; margin-top: 5px;"> <div>PI: Thomas C. Quinn</div> <div>Senior Investigator</div> <div>LIR, NIAID</div> </div> <div style="display: flex; justify-content: space-between; margin-top: 10px;"> <div>Others: Anthony S. Fauci</div> <div>Chief</div> <div>LIR, NIAID</div> </div>		
COOPERATING UNITS (if any) LCI, NIAID, S. James, W. Strober, M. Zeitz; Johns Hopkins University Medical Institution: M. Spence, F. Polk, J. Repke, J. Horn, E. Kappus, P. Rapoza, and H. Taylor.		
LAB/BRANCH Laboratory of Immunoregulation		
SECTION		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS <div style="text-align: center;">2</div>	PROFESSIONAL: <div style="text-align: center;">1</div>	OTHER: <div style="text-align: center;">1</div>
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between; margin-top: 5px;"> <div> <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input checked="" type="checkbox"/> (b) Human tissues </div> <div> <input type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> <u>Chlamydia trachomatis</u> is the most common sexually transmitted bacterial pathogen in the United States with 10 million cases annually. Studies have been carried out to further define the clinical spectrum of chlamydia infection, to develop rapid diagnostic assays, and to examine the pathogenesis of chlamydial infections in experimental animal models. In a study of 542 pregnant women, chlamydia was present in 16% and was significantly associated with the clinical findings of cervicitis, preterm delivery, low birth weight infants, and conjunctivitis. In a subsequent study of 505 pregnant women presenting for induced abortions, C. trachomatis, present in 17% of the patients, was also significantly associated with the development of postabortal endometritis. Newly developed diagnostic assays, such as immunofluorescent staining with monoclonal antibodies, and in situ DNA hybridization were shown to have a relatively high sensitivity and specificity for the detection of chlamydia. Development of a primate model of rectal C. trachomatis lymphogranuloma venereum (LGV) infection has aided in the study of the mucosal immune response to infection. Following rectal infection, there was reversal in the systemic T-cell lymphocyte populations and T-cell antigen specific responses to LGV were demonstrated in the peripheral blood and mesenteric lymph nodes, but not in the lamina propria. This failure to respond to C. trachomatis antigen within the mucosa correlated with persistence of C. trachomatis in tissue histiocytes as determined by immunofluorescence and In situ DNA hybridization. In summary, the above studies demonstrate the diverse clinical spectrum of C. trachomatis infection, and provide methods for the rapid screening of chlamydia and for studying the mucosal immune response to C. trachomatis. </p>		



LABORATORY OF INFECTIOUS DISEASES
1986 ANNUAL REPORT

Table of Contents

<u>Z01 AI</u>		
<u>Project Number</u>		<u>Page</u>
<u>Summary</u>		8-01
<u>Hepatitis Viruses Section</u>		
00308-05	<u>In Vitro</u> and <u>In Vivo</u> Studies of Hepatitis A Virus -- Purcell	8-31
00309-05	Structural and Antigenic Analysis of Hepatitis A Virus -- Feinstone	8-32
00310-04	Molecular Biology of Hepatocellular Carcinoma -- Feinstone (Terminated)	8-33
00311-05	Search for New Hepatitis Agents -- Ticehurst	8-34
00312-05	Clinical and Experimental Studies of Hepatitis B Vaccines -- Ticehurst	8-35
00313-04	Molecular Biology of Hepatocellular Carcinoma -- Purcell (Terminated)	8-36
00314-05	Woodchuck Virus -- Purcell	8-37
00316-05	The Delta Agent -- Purcell	8-38
00317-05	Biology of Non-A, Non-B Hepatitis Agents -- Feinstone	8-39
00319-05	New Approaches to <u>In Vitro</u> Propagation of Non-Cultivable Agents -- Purcell	8-40
00321-05	Development, Characterization, and Use of Cloned Hepatitis A Virus HAV cDNA -- Ticehurst	8-41
00370-04	Studies of Acquired Immune Deficiency Syndrome (Aids) -- Purcell	8-42
00404-03	Second and Third-Generation Hepatitis B Vaccines -- Purcell	8-43
00405-03	Inactivation of Hepatitis Viruses in Pooled Plasma Derivatives -- Purcell	8-44
00406-03	Molecular Structure of HAV: Genomic Sequence and Organization -- Cohen	8-45

00440-02	Construction of Full Length Hepatitis A Virus cDNA for Transfection -- Cohen	8-46
00473-01	Replication of Hepatitis A Virus <u>In Vivo</u> -- Cohen	8-47

Respiratory Viruses Section

00323-05	Structure of Parainfluenza Type 3 Virus Genome -- Collins	8-48
00324-05	Laboratory Studies of Myxoviruses -- Snyder	8-49
00325-05	Study of Respiratory Viruses in Primates -- Murphy	8-50
00326-05	Study of Respiratory Viruses in Volunteers -- Murphy	8-51
00327-05	Characterization of Parainfluenza and RS Virus Virus Surface Glycoproteins -- Coelingh	8-52
00344-05	Immunologic Factors in Respiratory Syncytial Virus (RSV) Disease -- Prince	8-53
00345-05	Immunity to RS Virus -- Prince	8-54
00368-04	Structural Analysis of Respiratory Syncytial Virus Genome -- Collins	8-55
00372-04	Respiratory Syncytial Virus Glycoproteins -- Collins	8-56
00457-02	Human Immune Response to Respiratory Syncytial Virus Infection or Vaccination -- Murphy	8-57
00474-01	Molecular Mechanisms Underlying Adenovirus Virulence -- Ginsburg	8-58

Molecular Viral Biology Section

00329-04	Expression of the Influenza A Virus Neuraminidase Glycoprotein from Cloned DNA -- Markoff (Terminated)	8-59
00332-04	Engineering the Genome of Influenza Virus -- Lai (Inactive)	8-60
00365-03	Functional Analysis of Signal Sequences in Influenza Hemagglutinin -- Lai (Inactive)	8-61
00366-04	Molecular Biology of Dengue Viruses -- Zhao	8-62
00369-03	Persistent Expression of Influenza Virus Polymerase Proteins from Cloned DNA -- Mackow (Terminated)	8-63

00407-03	Complementation of Influenza Mutants by Cloned Viral Genes -- Ryan	8-64
00408-03	Attempts at Allele Replacement of NA Gene of Influenza A Virus -- Markoff	8-65
00458-02	Genetic Variations Among Dengue Viruses -- Zhang	8-66
00459-02	Expression of Dengue Virus Proteins Using Baculovirus as a Vector -- Mackow	8-67
00460-01	Identification and Gene Mapping of Dengue Viral Antigens -- Makino (Terminated)	8-68
00475-01	Nucleotide Sequence of Genes for Dengue Virus Type 4 Non-Structural Proteins -- Mackow	8-69
00476-01	Engineering the Genome of Dengue Virus -- Breuning	8-70
00477-01	Expression of Dengue Viral Genes and Vaccinia Recombinant Virus -- Zhao	8-71

Epidemiology Section

00333-05	A Longitudinal Study of Viral Gastroenteritis in Infants and Young Children -- Kapikian	8-72
00334-04	Development of Monoclonal Antibodies to Rotavirus Proteins -- Midthun (Inactive)	8-73
00335-05	Rotavirus Reassortants: Genetics and Use in Rotavirus Vaccines -- Midthun	8-74
00338-05	Molecular Cloning and Sequencing of Rotavirus Genes -- Flores	8-75
00339-05	Isolation and Serotypic Characterization of Human and Animal Rotaviruses -- Hoshino	8-76
00340-05	Experimental Studies in Animals with Various Rotaviruses and Their Reassortants -- Hoshino	8-77
00341-05	Evaluation of Experimental Rhesus Rotavirus Vaccine in Infants and Children -- Kapikian	8-78
00342-05	Studies of Gastroenteritis Viruses by Electron Microscopy -- Kapikian	8-79
00343-05	The Role of Norwalk-Like 27nm Virus Particles in Viral Gastroenteritis -- Midthun	8-80
00346-05	Genetic Characterization of Rotavirus by Hybridization Techniques -- Flores	8-81

00410-02	Binding of Rotavirus to Cell Surface Receptors -- Askaa (Terminated)	8-82
00461-01	Serial Passages of Bovine and Rhesus Rotaviruses -- Askaa (Terminated)	8-83
00462-02	Rotavirus Vaccine Field Trial in Venezuela -- Flores	8-84
00463-01	Oligonucleotide Probes for Detection and Identification of Rotavirus Serotypes -- Glass (Terminated)	8-85
00448-01	Rotavirus Vaccine Trial in Umea, Sweden -- Glass (Terminated)	8-86
00449-01	Studies Examining a Protective Role for Breast-feeding in Rotavirus Diarrhea -- Glass (Terminated)	8-87
00450-02	Sequence of the Fourth Rotavirus Gene and Its Role in Virulence -- Gorziglia	8-88
00451-02	Use of Gene Reassortment to Study Rotavirus Gene Products -- Hoshino	8-89
00452-01	Characterization of Rotaviruses from Asymptomatic Human Neonatal Infections -- Hoshino (Terminated)	8-90
00453-01	Vaccinia Recombinant Containing Bovine Rotavirus Glycoprotein Gene -- O. Nakagomi (Terminated)	8-91
00454-01	Expression of Bovine Rotavirus Glycoprotein Gene in Mammalian Cells -- O. Nakagomi (Terminated)	8-92
00455-01	Expression of Rotavirus Neutralization Protein in Bacteria -- T. Nakagomi (Terminated)	8-93
00456-01	Assay of Coproantibodies to Rotavirus -- Tominack (Terminated)	8-94
00478-01	Development of Adenovirus Expression Vectors -- Flores	8-95
00479-01	Development of Monoclonal Antibodies to Proteins of Rotavirus -- Hoshino	8-96

ANNUAL REPORT

Laboratory of Infectious Diseases
National Institute of Allergy and Infectious Diseases
October 1, 1985 to September 30, 1986

INTRODUCTION

The mission of the LID continues to be definition of the cause and epidemiology of medically important viral diseases and development of means for their control. These efforts span a wide range of research activities extending from identification and antigenic characterization of viruses that cause acute disease of the respiratory and gastrointestinal tracts and the liver to basic molecular studies of viral structure, function and genome organization. Molecular biologic techniques are employed to elucidate pathogenesis of disease as well as to develop purified subunit antigens and attenuated viral mutants for use in prevention of respiratory, gastrointestinal and hepatic viral diseases.

During the past year significant progress was made in increasing our understanding of the genome organization and function of various gene products of dengue virus (a flavivirus), respiratory syncytial virus (a pneumovirus), parainfluenza type 3 virus (a paramyxovirus), rotavirus (a member of the family reoviridae) and hepatitis A virus (a picornavirus). Each of these viruses is the cause of a medically important disease. Furthermore, a new strategy for immunization against respiratory syncytial virus (RSV) was developed using vaccinia virus recombinants bearing the gene for the F or G surface glycoprotein of RSV. Initial studies of these recombinants in experimental animals were highly successful. Continued clinical evaluation of the rhesus rotavirus (RRV) candidate live vaccine strain provided a basis for optimism that this approach, possibly broadened to include RRV-human rotavirus reassortants bearing the major neutralization protein of a human rotavirus, may yield a safe and effective vaccine for prevention of rotavirus diarrhea of early life.

During the past year we were fortunate to have Dr. Harold Ginsberg, until recently Chairman of Microbiology, College of Physicians and Surgeons, Columbia University, join our staff for a one year sabbatical under an IPA appointment. Dr. Ginsberg has rekindled our interest in adenoviruses by his elegant studies of the molecular basis of adenovirus virulence in an experimental host.

SUMMARY

HEPATITIS VIRUSES

Hepatitis A Virus (HAV)

The complete nucleotide sequence of wild-type HAV strain HM-175 was determined. The genome is 7478 nucleotides long, is followed by a poly A tail, and encodes a polyprotein of 2227 amino acids. On the basis of amino acid homology with other picornaviruses, putative cleavage sites for the polyprotein have been identified. The molecular weights of outer capsid proteins VP1, VP2, and VP3 predicted from the sequence data agree with those determined by biophysical methods. HAV proteins 2C and 3D are the most homologous with other picornaviruses and the putative active enzyme sites present on 3C and 3D of

other picornaviruses are also found in the same locations on HAV. Comparison of the sequence of wild-type HM-175 with that of a cell culture adapted strain (Najarian, PNAS 1985) revealed 624 nucleotide differences (91.7% identity), but there were only 34 amino acid differences (98.5% identity) (Cohen, Ticehurst, Feinstone, Purcell).

Cloned cDNA was used as a hybridization probe for detecting HAV RNA in tissue culture, serum, and fecal specimens. Hybridization experiments also demonstrated that probes taken from any region of the HAV genome do not hybridize to RNA or cloned cDNA from a variety of other picornaviruses. In addition, from analysis of the complete nucleotide and predicted amino acid sequences of this genome and comparison with sequences from other picornaviruses, it appears that HAV has the genome organization of a typical picornaviral but its sequences are widely divergent from other picornaviruses and hence it should be classified separately from other picornaviral genera (Ticehurst, Cohen, Feinstone, Purcell). The viral protein that binds to the 5' terminus of genomic RNA, i.e., HAV VPg, was demonstrated directly for the first time using antibodies directed against a synthetic peptide representing the VPg amino acid sequence predicted from cloned cDNA. These antibodies reacted with the covalent HAV RNA-protein (VPg) complex (Weitz, Cohen, Ticehurst).

Six cDNA clones which together span the entire HAV genome were isolated and ligated together to form a single clone in pBR322 which was thought to represent full length HAV cDNA. Transfection of both tissue culture cells (in vitro) and marmosets (in vivo) with this plasmid failed to initiate infection. Fine structure mapping of the HAV cDNA indicated that about 40 base pairs had been deleted during the ligation process. The deletion was repaired, but transfection of marmosets and tissue culture cells still failed to generate HAV. An additional modified construct, differing by two nucleotides thought to be important for infectivity, also failed to generate HAV in marmosets. The entire full length construct was then sequenced and compared with the sequence of the parent clones used to create the construct. This comparison did not reveal any differences. The cDNA was then inserted into an RNA transcription vector and plus strand RNA was synthesized in vitro from the cDNA. Transfection of marmosets with this RNA also failed to initiate HAV infection. Currently, plus strand HAV RNA is being synthesized in vitro from minus strand HAV RNA using poliovirus RNA polymerase (Cohen, Ticehurst, Feinstone, Purcell).

HAV which has been successfully adapted to growth in African green monkey kidney tissue culture is predominantly cell-associated and does not produce cytopathic effects (CPE). After 10 to 20 serial passages in tissue culture HAV was attenuated for chimpanzees. Significantly, attenuated HAV did not revert to virulence during 3 serial passages through chimpanzees. The tissue culture-adapted HAV was then thrice cloned by terminal dilution and virus suspensions representing passages 20 and 30 were characterized in chimpanzees and marmosets. These virus suspensions were attenuated compared to parental wild-type virus. Immunization of chimpanzees with attenuated HAV protected most but not all vaccinated animals from challenge with parental HAV. Attenuated HAV suspensions at the 20th or 30th tissue culture passage are currently being considered for phase I clinical trials to be performed in collaboration with the Walter Reed Army Institute of Research (WRAIR) and SKF RIT, an American-Belgian pharmaceutical company (Karron, Feinstone, Purcell).

Hepadnaviruses - (a) Hepatitis B Virus (HBV)

Hepatitis B virus (HBV), although classified as a double-stranded DNA virus, has been shown to replicate by a mechanism similar to that of the retroviruses which involves reverse transcription of RNA. Computer analysis indicated that the most highly conserved sequence on the HBV genome, positioned at or near the initiation site for first strand DNA synthesis, is homologous over 67 nucleotides to the U5 region of retroviral long terminal repeats. Contained within a highly conserved (i.e., 90%) 16 nucleotide sequence a heptanucleotide sequence CCTTGGG is 97% homologous among 27 retrovirus and hepadnavirus isolates. In addition, the most conserved HBV protein, the core (or nucleocapsid) protein, shares 41% homology over 98 amino acids with the carboxyl-terminal region of the p30 gag nucleocapsid protein of type C retroviruses. Coupled with the shared homology within the viral polymerases, discovered by others, these observations suggest that HBV and retroviruses shared a common ancestor in relatively recent evolutionary time. The genetic relatedness of hepadnaviruses to retroviruses may help explain the mechanism of the integration of HBV DNA in the human genome. This is important because infection with HBV has been linked to development of hepatocellular carcinoma in later life, and HBV DNA has been found to be integrated into liver tumor DNA. Previous studies by others have identified numerous retrovirus-like genetic elements integrated into the human genome. Although not infectious, these elements may have evolved from infectious human retroviruses related to the murine leukemia/sarcoma type C retroviruses. In addition to possessing homology to the type C retroviruses, these elements share substantial nucleotide sequence homology with HBV at the same sites that are homologous among the hepadnaviruses and retroviruses as well as additional sites. Thus, because the endogenous DNA elements and the hepadnavirus family share nucleotide sequence homology: (1) these elements may prove to be an evolutionary link between the retrovirus and hepadnavirus families and (2) these elements may provide sites for homologous recombination which allow the HBV genome to integrate into human chromosomal DNA (Miller).

Clinical testing and characterization of one lot of plasma-derived HBV vaccine prepared by the NIAID have been completed. The vaccine was highly immunogenic, safe and well tolerated when tested in healthy persons ranging from infants to adults. Results from an efficacy trial in Asia indicate that the vaccine effectively prevents transmission of HBV from infected mothers to their infants. The sera from these infants are being analyzed for responses to specific HBsAg epitopes in an attempt to identify those sites that are most important in resistance (Ticehurst, Purcell).

(b) Woodchuck Hepatitis Virus (WHV)

The woodchuck hepatitis virus (WHV) is taxonomically and serologically related to HBV. Infection with each of these viruses is associated with acute and chronic hepatitis and hepatic cell carcinoma in their respective hosts and these associations appear to be etiological in nature. The WHV/woodchuck model system provides a convenient means of studying the relationship between virus and host in the oncogenic process. Genetic relationships between WHV and HBV suggest a common origin and hence new insights into the molecular biology and pathogenesis of the former virus have relevance to our understanding of type B hepatitis and HBV-associated human hepatocellular carcinoma. Genetically altered WHV genomes, constructed by recombinant DNA techniques are currently being studied in woodchucks in order to determine the biological significance of individual gene products (Miller, Purcell).

Epidemic Non A, Non B Hepatitis Viruses

New hepatitis agents continue to be recognized. Recently, a form of epidemic hepatitis which occurs frequently in India could not be associated with any of the recognized hepatitis viruses. Previous attempts to transmit an agent from acute-phase clinical specimens to primates were partially successful. Transient low-level liver enzyme elevations and histopathologic changes consistent with hepatitis in liver biopsies were observed in some animals, but this was not uniform, and attempts to serially transmit an agent in chimpanzees and marmosets also produced irregular results. Efforts to identify viral agent(s) in recently obtained clinical specimens from India, Algeria and the USSR include attempted transmission in primates, immune electron microscopy, and hybridization with cloned picornavirus cDNA. Although an etiologic agent has not been identified in experimental animals and none of the specimens has hybridized with probes that detect a wide range of picornaviruses including HAV, several distinct virus-like particles have been visualized. Attempts to define a serologic relationship between epidemic non A, non B hepatitis and one or more of these particles are in progress (Ticehurst, Kapikian, Purcell).

Blood Borne Non A, Non B Hepatitis Viruses

The transmission of viral hepatitis, predominantly non A, non B, by plasma derivatives continues to be an important medical problem that has been made more urgent by the similar pattern of transmission of the agent of acquired immune deficiency syndrome by blood products. Methods of inactivating hepatitis viruses in plasma derivatives while retaining the biological potency of the products have been evaluated and the most promising technique appears to be extraction of plasma derivatives with H₂O saturated chloroform. This technique inactivates the Hutchinson strain of non A, non B hepatitis and another strain which has been identified as a contaminant of clotting factors VIII and IX. These viruses as well as the AIDS retrovirus are inactivated without significantly altering the biologic activity of clotting factor VIII. Our studies indicate that chloroform is potentially useful for the inactivation of lipid-containing viruses in plasma products and that it can be used either as a two-phase water/chloroform preparation for treating plasma products in solution or as a water-saturated single-phase chloroform preparation for treatment of lyophilized products (Purcell).

Delta Virus (HDV)

The delta virus (HDV) is a transmissible hepatitis virus that is defective and requires co-infection with HBV for its own synthesis. The agent has a small RNA genome ($10^{5.7}$ daltons) that is encapsidated together with delta antigen within a coat of HBs Ag that is provided by its helper virus, i.e., HBV. HDV was discovered in 1977 in Italy, where it is endemic. Subsequently, it was shown during studies in LID to be an infectious agent which requires coinfection with HBV for its replication in vivo. Evidence for infection with HDV is found most frequently in carriers of HBV who are repeatedly exposed to blood (hemophiliacs, illicit drug users, etc.). Sensitive assays for HDV infection were developed in LID and used to evaluate experimental infection of HBV-carrier chimpanzees. Infection of humans or chimpanzees with HDV results in very severe hepatitis. HDV has also been experimentally transmitted to woodchucks chronically infected with the WHV, a hepatitis virus similar to HBV. The chimpanzee and woodchuck thus provide animal model systems for more detailed characterization of the medically important HDV (Purcell).

Attempts to clone the genome of the delta virus, in collaboration with Dr. J. Gerin (Georgetown University), have been partially successful. A portion of the putative delta genome has been cloned and hybridization experiments between HBV and delta virus DNA probes failed to reveal a relationship between HBV DNA and delta RNA. Delta cDNA has also been used as a probe for the detection of HDV RNA in clinical specimens. HDV RNA was detected in over half of the patients with either acute or chronic HDV infection. Its detection correlated with other markers of HDV infection, including HDAg in liver biopsies and IgM anti-HD in serum. Thus, the cDNA provides a sensitive marker of active HDV replication (Purcell).

Dr. Yohko Shimizu, a former visiting associate, has generated monoclonal antibodies that react with a cytoplasmic antigen in hepatocytes from chimpanzees experimentally infected with non-A, non-B hepatitis virus. In collaborative studies with Dr. Shimizu it was shown that a presumably identical antigen is present in hepatocytes from chimpanzees experimentally infected with HDV. Extensive evaluation of experimentally infected chimpanzees confirmed that this antigen did not result from contamination of the HDV inoculum with non A, non B hepatitis virus. Instead, it appears that the "Shimizu" antigen is a host protein which is induced by infection with either non A, non B virus or delta virus (Purcell).

RESPIRATORY VIRUSES

Respiratory Syncytial Virus (RSV)

RSV is the major etiologic agent of severe viral lower respiratory tract disease during early life and the need for effective immunoprophylaxis has been clearly established in studies performed throughout the world. During the 30 years since the first strains of RSV were recovered from humans much has been learned about its epidemiology and recently, its genome organization and gene products have been defined in considerable detail.

RSV: Analysis of Genome

Previously, complete cDNAs and complete nucleotide sequences were obtained for nine of the ten known viral mRNAs of human RSV strain A2. During the past year synthetic oligodeoxynucleotides were used to direct dideoxynucleotide sequencing of intergenic and flanking regions in the viral genome (vRNA). Comparison of the intergenic and flanking sequences with the complete mRNA sequences established unambiguously the 3' to 5' order of the nine genes on vRNA, 3'-NS1-NS2-N-P-M-SH-G-F-22K-L. Each gene was immediately followed (in genome-sense) by an oligo U tract of 4-7 residues that probably direct synthesis of poly A tails of the mRNAs by a reiterative copying mechanism. The intergenic regions varied in length from 1 to 52 nucleotides and displayed no obvious sequence conservation except that in all cases the last nucleotide (vRNA-sense) was an A residue. The sequence of the first 1010 nucleotides of the L gene was determined by dideoxynucleotide sequencing of vRNA. Mapping and sequencing of the 5' end of the L mRNA established that, unexpectedly, the L gene overlaps its upstream neighbor, the 22K gene. Specifically, the first 68 nucleotides of the L mRNA are identical to the last 68 nucleotides of the 22K mRNA, and are encoded by the same vRNA sequence. This genetic arrangement is without precedent among negative strand RNA viruses. Finally, cDNA cloning and sequence analysis have been initiated for two additional RS viruses, the Long and 18537 strains. These have been shown by others to be distinct from the previously characterized A2 strain on the basis of reactivity with a panel of

monoclonal antibodies to proteins of the envelope and nucleocapsid. Information on the structural and antigenic heterogeneity of RS viruses is required for the design of effective RSV vaccines. Preliminary sequence data indicates that the 18537 strain is relatively more distinct from strain A2 than is the Long strain, consistent with the results for monoclonal antibody reactivity. The available information also supports the hypothesis that the G protein is structurally more diverse than the other virion components (Collins, Johnson, Olmsted, Spriggs).

RSV: Characterization of Glycoproteins and Their Possible Usefulness In Immunoprophylaxis

Previously, complete sequences for the mRNAs encoding the RSV G and F glycoproteins were determined from cDNA clones (annual reports, 1984, 1985, and Satake et al., 1985). During 1985-6 vaccinia virus recombinants (vaccinia-F and vaccinia-G) that express the individual RSV glycoproteins were constructed in collaboration with Dr. B. Moss (LVD). Cotton rats were then infected with vaccinia-F or vaccinia-G to evaluate the relative contributions of the individual RSV glycoproteins to host immunity. Both recombinants induced high levels of serum RSV antibodies that neutralized RSV infectivity. Vaccinia-G induced substantial resistance in the lungs to RSV infection but resistance in the nose was not detected. Vaccinia-F induced essentially complete resistance to RSV infection in the lungs and partial resistance in the nose. Significantly, the serum neutralizing antibody titers induced by vaccinia-F were slightly higher than those induced by RSV infection of the respiratory tract, and were 6-fold higher than those induced by vaccinia-G. Thus, the F glycoprotein appears to be more effective than the G glycoprotein in stimulating host immunity (Olmsted, Collins, Prince, Murphy, Chanock).

Much remains to be done before vaccinia-RSV recombinant viruses can be evaluated in clinical trials. For example, it must be shown that the recombinants induce a high level of serum neutralizing antibodies in primates and that immunization of these species stimulates effective resistance to RSV infection in the lower respiratory tract. Also, immunogenicity and protective efficacy in primates must be established for vaccinia recombinants constructed from a parental vaccinia virus which has been licensed for use in man. Duration of effective immunity in the lungs of primates must be defined to determine whether primary immunization would be expected to protect infants and young children during their period of greatest vulnerability, i.e., the first two years of life. If experimental studies indicate that effective lower tract immunity wanes after 6 months to a year, the feasibility of booster immunization must be explored. Finally, the possibility of immunosuppression of response to RSV glycoproteins must be investigated by performing immunogenicity and protective efficacy studies in primates with a range of pre-existing serum neutralizing antibodies similar to those present in young infants, the proposed recipients of an RSV vaccine.

RSV F and G cDNAs have also been expressed in tissue culture using recombinant SV40 viruses. In conjunction with site-directed mutagenesis, these studies will define amino acid sequences that are important in protein function (Olmsted, Collins, Markoff).

Monoclonal antibodies to the fusion and G surface glycoproteins of respiratory syncytial virus have been produced and are being characterized

prior to selection of antigenic variants, epitope mapping, and sequence analysis of variants (Beeler, Coelingh).

RSV: Immunology and Immunopathology

One of the most unusual aspects of RSV is that the highest incidence of serious disease occurs during the first 6 months of life. The peak occurs during the second and third months but there is a relative sparing from serious disease during the first month. The later observation suggests that a high level of maternally-derived serum neutralizing antibodies protect the lower respiratory tract. Such a protective effect of a high level of serum neutralizing antibodies is supported by studies of experimental RSV infection in cotton rats. The amount of passively acquired serum RSV neutralizing antibodies required to protect the respiratory tract against infection was studied in infant cotton rats which were inoculated intraperitoneally with various dilutions of a single pool of sera derived from cotton rats convalescent from RSV infection. After 24 hours, these animals were inoculated with RSV intranasally and their upper and lower respiratory tracts were assayed for level of virus replication 4 days later. Virus replication in the respiratory tract was suppressed in cotton rats which had a serum neutralizing antibody titer of 1:100 or greater. Resistance was significantly greater in the lungs than in the nose. Complete or almost complete resistance in the lungs was observed in cotton rats with a serum neutralizing antibody titer of 1:380 or greater. The level of RSV neutralizing antibodies in serum required to confer significant resistance to infection in the cotton rat was similar to the level of maternally-derived serum RSV antibodies possessed by human infants less than 2 months of age who exhibit relative resistance to RSV disease compared to infants 2 to 6 months of age. Although high levels of serum neutralizing antibodies are relatively ineffective in protecting the nose of cotton rats, infection of this region by RSV provides significant or complete resistance in the upper respiratory tract for 6-12 months. These observations suggest that the upper tract is protected primarily by local immunity such as that conferred by IgA RSV antibodies. Previous studies performed in adult volunteers support this interpretation (Prince, Murphy, Chanock).

Human convalescent antiserum to respiratory syncytial virus (RSV) administered intraperitoneally to cotton rats prior to RSV challenge also provided near-complete protection from pulmonary infection. In addition, antiserum given subsequent to viral challenge at the peak of virus replication, reduced pulmonary viral titer 10^3 or greater within 24 hours. Sandoglobulin, a preparation of purified human IgG with high titer of RSV neutralizing antibodies, produced the same effects as convalescent antiserum. Sandoglobulin was absorbed rapidly and produced a significant therapeutic reduction in pulmonary virus titer within 3 hours. The degree of reduction of virus in pulmonary and nasal tissues was directly proportional to the titer of neutralizing antibodies attained in the serum of the recipient cotton rat and the extent of reduction was always greater in the lungs than the nose (Prince, Murphy, Chanock).

Studies in the owl monkey showed that the intravenous administration of human RSV antibodies at the time of maximum viral shedding reduced pulmonary viral titer an average of $10^{-1.5}$ within 48 hours, and cleared detectable virus from 75% of the animals. In spite of relatively high doses of antibody (3 gm/Kg) the treatment was well tolerated and did not exacerbate pulmonary disease. On the basis of these two studies, clinical trials of antibody

therapy in infants hospitalized with RSV bronchiolitis and/or pneumonia are currently in progress at Children's Hospital National Medical Center and Walter Reed Hospital (Prince, Murphy, Chanock).

Therapy of RSV lower respiratory infection in the cotton rat can also be achieved by administration of RSV antibodies directly into the lungs. The amount of antibodies required for a therapeutic effect is one three hundredth that needed when RSV antiserum is administered parenterally suggesting that aerosol administration of human RSV antibodies should be evaluated for its therapeutic effect in a clinical trial involving infants with serious RSV bronchiolitis or pneumonia (Prince, Murphy, Chanock).

The potentiation of RSV lower respiratory tract disease by prior inoculation of formalin-inactivated RSV vaccine, which occurred during clinical trials of the vaccine 20 years ago, was reproduced in the laboratory for the first time. Potentiation of pulmonary pathology was observed when cotton rats (*Sigmodon hispidus*) previously immunized with formalin-inactivated RSV vaccine underwent a RSV infection of their respiratory tract. Within 24 hours after infection with RSV, immunized cotton rats developed exaggerated pulmonary lesions that reached a maximum by day 4. Histologically, the lesions resembled an experimental pulmonary Arthus reaction. An action of formalin on RSV appears to be responsible for this effect, because parenteral immunization with live virus or virus incubated at 37°C in the absence of formalin did not induce enhanced immunopathology. Epitopes on the fusion (F) and/or attachment (G) RSV surface glycoproteins involved in inducing neutralizing antibodies were modified by formalin resulting in reduction or ablation of a neutralizing antibody response. Nonetheless, cotton rats inoculated parenterally with formalin-inactivated virus developed a high level of F and G antibodies measurable by an enzyme-linked immunosorbent assay (ELISA) indicating that most of the epitopes not involved in neutralization were not altered by formalin. Although the sites on the F and G glycoproteins at which formalin acts to produce its disease-enhancing effect have not been identified, it is clear that formalin-treated RSV stimulates an unbalanced immune response in which an unusually large proportion of the induced antibodies are directed against nonprotective epitopes on the viral surface glycoproteins. Consequently, effective resistance is not provided, but a high concentration of antibodies that bind to RSV proteins, such as the F and G glycoproteins, is present in the serum and is available to form complexes with viral antigens that are produced during infection. Also, the host may be primed for an accelerated immune response to nonprotective antigenic sites when infection occurs. Staining of lung sections from vaccinated, infected animals showed abundant deposits of IgG and C₃, indicating a major role for a type III (Arthus) immunopathologic reaction (Prince, Murphy, Chanock).

RSV: Immunologic Response of Young Infants To RSV Infection Or Immunization With Formalin-inactivated Virus

Young infants (<8 months of age) have a diminished serum antibody response to RSV infection as measured by F glycoprotein or G glycoprotein-specific ELISA or by neutralization of virus infectivity. Age primarily affects the response to the F glycoprotein and pre-existing antibody suppresses the response to the G glycoprotein. Infants and children less than two years of age primarily see the heavily glycosylated G glycoprotein as a protein antigen, i.e., they mount ELISA IgG1 and IgG3 subclass responses to the G glycoprotein. Adults, however, generate an IgG2 response equivalent to their IgG1 response. Thus, the G

glycoprotein can be seen by the immune system as a protein and/or carbohydrate antigen. During infancy the G glycoprotein is seen primarily as a protein antigen and this response is suppressed by maternally-derived serum antibodies (Murphy).

Infants and children immunized with formalin-inactivated RSV vaccine 20 years ago developed titers of ELISA antibodies equivalent to those of individuals infected with RSV, but the neutralizing activity of these antibodies was low. These findings confirm previous observations made in cotton rats immunized with formalin-inactivated RSV and support the view that treatment of RSV with formalin preferentially alters the epitopes of the F and/or G glycoproteins that stimulate neutralizing antibodies with the result that the immune response to vaccine consists largely of "nonfunctional" (i.e., non-neutralizing) antibodies (Murphy, Prince, Chanock).

Antigenic polymorphism among RSV strains was recognized in cross neutralization tests performed over 20 years ago in LID. Recently the two RSV antigenic subgroups were defined in greater detail by others using monoclonal antibodies. The major share of the antigenic dimorphism initially recognized by the neutralization assay appears to be linked to variation in the G glycoprotein. In tests performed in LID on convalescent sera of infants undergoing primary infection with RSV the extent of reciprocal reduction in heterologous neutralization observed for the antigenic subgroups was approximately 3-fold. This difference is considerably less than that which occurs with viruses belonging to different influenza A virus subtypes. It appears that the differences among the most divergent RSV strains are equivalent to those which exist between successive antigenic variants within an influenza A virus subtype. This degree of antigenic dimorphism may contribute to the occurrence of initial reinfection but its effect can hardly explain the major aspects of RSV epidemics and patterns of disease. In any case, it would be prudent to include both antigenic variants in a RSV vaccine (Murphy, Chanock).

Parainfluenza Type 3 Virus (PIV3)

Analysis of Genome and Its Gene Products

cDNA clones of human parainfluenza type 3 virus (PIV3) mRNAs encoding the hemagglutinin-neuraminidase (HN) glycoprotein, fusion (F) glycoprotein, major nucleocapsid protein (NP), nucleocapsid phosphoprotein (P), and matrix (M) protein were constructed, identified and characterized. The P mRNA contains a second, overlapping open reading frame that encodes the nonstructural (C) protein. Complete nucleotide sequences have been determined for the HN, F, NP, P+C and M mRNAs. Synthetic oligonucleotides were used to direct dideoxynucleotide sequencing of gene junctions in PIV3 genomic RNA (vRNA). During the sequencing of vRNA, a seventh viral gene was detected and was identified as the L gene by RNA blot hybridization. The order of the six PIV3 genes on vRNA is 3'-NP-P+C-M-F-HN-L. The five intergenic regions consist of the trinucleotide 3'-GAA. These studies demonstrate that PIV3 encodes six mRNAs (NP, P+C, M, F, HN and L) that encode seven proteins (NP, P, C, M, F, HN, and L). Preliminary studies on the expression of the F and HN genes by recombinant vaccinia and SV40 viruses are in progress. These studies entailed the construction of new SV40 vectors and the development of a new strategy for rapid mutagenesis and expression (Spriggs, Collins).

Hemagglutinin-Neuraminidase Glycoprotein: Fine Structure Antigenic Analysis

The operational epitope and functional maps of the PIV3 hemagglutinin-neuraminidase (HN) protein have been expanded. Monoclonal antibodies (mAbs) to the HN protein define 14 operationally unique epitopes which are organized into 5 topographically non-overlapping antigenic sites (A,B,D,E, and F) and one bridging site (C). MABs to sites A,B, and C inhibit hemagglutination and infectivity, and several site A mAbs also inhibit sialidase activity. MABs to sites D, E, and F do not inhibit any known biological activity and react with all but 1 of 37 clinical PIV3 isolates examined, which is in contrast to mAbs to more variable epitopes in sites A,B, and C. Sequence analysis of HN genes of 16 mAb-resistant antigenic variants indicate the HN epitopes are located in hydrophilic stretches of amino acids. Computer analysis predicts these amino acids to form hydrophilic loops which connect B-sheet structures. Antigenic variants of human PIV3 selected with mAbs which cross-react with the bovine PIV3 have amino acid substitutions in residues which are conserved in the primary structure of the parental human and bovine PIV3 strains (Coelingh, Murphy, Olmsted, Collins).

Resistance To Human PIV3 Induced By Bovine PIV3

Bovine PIV3, which shares certain epitopes in 3 of the 6 HN antigenic sites with human PIV3, is attenuated for chimpanzees and induces a high level of resistance to infection with human PIV3 in squirrel monkeys. These observations suggest that the bovine PIV3 may prove to be useful in immunization against human PIV3 disease during infancy and early childhood. There is a clear need for an effective PIV3 vaccine because this virus is second only to RSV as a cause of severe viral lower respiratory tract disease during infancy and early childhood (Coelingh, Prince, Murphy).

Influenza A Virus

Attenuation Of Human Influenza A Viruses By Transfer Of Avian Influenza A Virus Genes: Further Studies In Monkeys

We previously demonstrated that the M and NP genes of the avian influenza A/Mallard/NY/78 donor virus were the major genetic determinants of attenuation of this virus (and its reassortants) for squirrel monkeys. Single gene substitution reassortant viruses containing seven genes from the human influenza A/Washington/80 H3N2 wild type virus and one "internal" gene (i.e., a gene which codes for a non-surface protein) from another attenuated avian influenza donor virus, A/Pintail/Alberta/119/79, were prepared in order to analyze the contribution of each of its "internal" genes to the restriction of this avian virus in the lungs of squirrel monkeys. Five of the possible six single "internal" gene reassortants were isolated and evaluated in squirrel monkeys for their level of replication in the nasopharynx and trachea. In addition, ten other reassortants containing two or more avian influenza A virus "internal" genes were evaluated. The nucleoprotein (NP) gene, but not the M gene, of the A/Pintail/Alberta/119/79 avian influenza A virus specified attenuation in monkeys. The PB2 gene also contributed to attenuation, but the restriction of replication specified by this gene was modified by the presence of other avian or human influenza A virus genes. A reassortant lacking the attenuating avian influenza virus NP and PB2 genes, but containing the other four avian influenza A virus "internal" genes was also attenuated in monkeys. Thus, genes other than NP and PB2 can also contribute to the attenuation phenotype of the A/Pintail/Alberta/119/79 virus and its reassortants (Snyder, Murphy).

Infectivity Of An Avian-Human Influenza A Virus Reassortant For the Upper and Lower Respiratory Tract of Squirrel Monkeys

The infectious dose⁵⁰ of an avian-human influenza A reassortant virus (bearing six "internal" avian influenza A virus genes) for squirrel monkeys was essentially the same whether delivered by small particle (<5µm) aerosol or by intranasal instillation. This suggests that the aerosol route of administration of influenza A viruses that are restricted in their replication in the lungs has little advantage over the intranasal route (Snyder, Murphy, Chanock).

Sequence and Biologic Analysis of Attenuating Avian Influenza A Virus Genes

Studies with reassortant viruses which contained human influenza A virus surface antigens from the A/Washington/80 (H3N2) virus and one or more "internal" genes from the avian A/Pintail/Alberta/119/79 virus demonstrated: (1) a particular constellation of polymerase genes specifies a host range dependent restriction of replication in MDCK but not chick kidney tissue culture, (2) the ability to replicate at 42°C is a complex, multigene determined phenotype, and (3) the NP gene is the major contributor to attenuation in monkeys (Snyder, Buckler-White, Murphy).

Comparison of the nucleotide and amino acid sequences of the NP genes of the avian A/Mallard/NY/78 with those of two other avian and three human influenza A viruses indicated the existence of separate avian and human classes of NP genes with separate evolutionary patterns. The rate of evolution of human influenza A virus NP genes in the first several years immediately following the emergence of a new subtype in 1968 was increased compared with other intervals (Buckler-White, Snyder, Murphy).

Attenuation of Avian-Human Influenza A Reassortant Viruses For Humans.

It was previously demonstrated that live influenza A reassortant viruses which derived their genes coding for surface glycoproteins from the human influenza A/Washington/89/80 (H3N2), A/Korea/1/82 (H3N2), or A/California/10/78 (H1N1) virus and their other six genes (i.e., "internal" genes) from the avian influenza A/Mallard/NY/6750/78 (H2N2) virus were consistently attenuated, safe and immunogenic in adult volunteers and that infection with the influenza A/Korea/82 x A/Mallard/NY/78 virus induced resistance against illness produced by challenge with wild-type A/Korea/82 virus. Because initial clinical studies with the avian influenza A/Mallard/NY/78 donor virus suggested that it might be overattenuated, we undertook the evaluation of two additional avian influenza A donor viruses which replicated to higher titer in the lower respiratory tract of squirrel monkeys. Reassortant viruses derived from the avian influenza A/Pintail/Alberta/119/79 (H4N6) donor virus were less infectious than similar reassortant viruses derived from the avian influenza A/Mallard/NY/78 donor virus suggesting that reassortants derived from the former virus were overattenuated in adults. For this reason we evaluated a reassortant virus derived from another donor, the avian influenza A/Mallard/Alberta/88/76 (H3N8) virus, which although restricted, replicated to a moderately high titer in the lower respiratory tract of squirrel monkeys. Studies in susceptible adult volunteers indicated that the six "internal" genes of avian influenza A/Mallard/Alberta/88/76 donor virus attenuated the wild-type human influenza A/Korea/1/82 virus for humans. In terms of attenuation, safety, restriction of virus replication and immunogenicity, this avian-human influenza A reassortant virus was similar to the previously studied human-avian influenza A reassortant

viruses derived from the avian influenza A/Mallard/NY/78 donor virus (Snyder, Murphy).

Analysis Of "Internal Genes" Of The Human Influenza A Cold Adapted (ca) Donor Virus In The Laboratory and In Adult Volunteers

Studies in hamsters, ferrets and tissue culture of single gene substitution reassortant viruses derived from the human influenza A/Ann Arbor/6/60 ca donor virus confirmed the role of polymerase PA in specifying the ca phenotype and that of the polymerase PB2 in specifying the ts phenotype. The polymerase PA and the M genes appear to play a major role in attenuation. The mechanism of attenuation by the M gene is independent of the ts or ca phenotype (Snyder, Murphy).

Studies in adult volunteers of reassortant viruses which contain a single "internal" gene derived from the influenza A/Ann Arbor/6/60 ca donor virus and all other genes from the influenza A/Korea/82 virus confirm our hypothesis that the polymerase PA of the ca donor virus plays a major role in the ca and attenuation phenotypes. These studies also indicate that attenuation specified by the polymerase PA is due to a mechanism other than temperature sensitivity (Snyder, Murphy).

Significant Viable Deletion Mutation In NS Gene: Attenuation and Genetic Stability

A viable reassortant virus (CR43-3) isolated by Dr. Maassab of U. of Mich. from the mating of human influenza A/Alaska/6/77 (H3N2) virus x A/Ann Arbor/6/60 (H2N2) ca donor virus sustained a spontaneous in-phase deletion of 36 bases in the region the NS gene which codes for the NS1 protein. The mutant NS gene is otherwise identical to that of the influenza A/Alaska/77 wild-type parent virus and hence was derived from this virus. All of the other genes except those coding for the surface glycoproteins of the reassortant were derived from the ca parent virus. Although both parental viruses grow well in both canine kidney tissue culture (MDCK) and chick kidney tissue culture (PCKC) and in eggs, the CR43-3 reassortant virus does not produce plaques on MDCK cells. When reassortant viruses were produced which contained various combinations of CR43 and A/Alaska/6/77 wild type genes, the host range restriction of replication was found to require the mutant NS gene. In order to evaluate the biological effects of the viable deletion mutation in the NS gene on a wild type background, a reassortant virus (clone 143-1) was isolated which contained the mutant NS gene in association with seven genes of the wild type A/Alaska/6/77 virus. This reassortant did not express a host range restriction of replication in cell culture indicating that interaction of the mutant NS gene with one or more ca genes is required for expression of this phenotype. However, the reassortant exhibited the ts phenotype. The reassortant was only moderately restricted in replication in the respiratory tract of hamsters and chimpanzees. Significantly, loss of the ts phenotype occurred after replication of the reassortant in hamsters and chimpanzees. This was an unanticipated finding because of the relatively large size of the NS gene deletion (i.e., 36 nucleotides). Nonetheless, it is consistent with the ability of negative strand RNA viruses to develop second site mutations which allow them to escape from the restriction imposed on their replication by previous missense mutations. It will be of considerable interest to define the second site mutation(s) responsible for loss of the ts phenotype specified by the NS gene deletion (Snyder, Murphy, Chanock).

Attempts At Allele Replacement Of Influenza A Virus Neuraminidase (NA) Gene
By Transcripts From Cloned Influenza A NA cDNA: Influenza A Virus
"Gene Rescue"

During the past few years recombinant DNA technology has been employed for the purpose of developing a more effective live influenza virus vaccine. The intent was to develop a "donor" influenza virus vaccine strain by creating specific mutations in a gene or set of genes coding for viral proteins other than the surface antigens. We sought mutations that confer attenuation, lack of transmissibility, and genetic stability during multiple rounds of virus replication in the human respiratory tract. Current technology does not permit us to produce specific, potentially stable deletion or point mutations in the virion RNA of influenza virus. Since these sorts of mutations are readily created in DNA, we have had a longstanding interest in developing techniques for rendering cloned full-length DNA copies of influenza genes fully active biologically, i.e., capable of being rescued by an infectious influenza virus and capable of functioning in such a manner that virus bearing the rescued gene is able to infect and replicate. Thus, RNA transcripts produced intracellularly or *in vitro* from influenza DNA would be recognized during replication or packaging of influenza virions and incorporated as a functional gene into progeny virus. If this could be achieved, it would then be possible to rescue influenza DNA bearing specifically engineered mutations.

Previously we failed to rescue a full-length DNA copy of the influenza HA or NA gene cloned into the late region of an SV40 expression vector in either (+) or (-) orientation with respect to mRNA transcription. Analysis of this vector system suggested that the experiments did not succeed because the RNA transcripts produced in infected cells contained SV40 sequences at the 5' end and SV40 chain termination signals plus poly-A at the 3' end. Influenza viral RNAs have unique conserved sequences at their 3' and 5' ends which are probably necessary for recognition by viral RNA polymerases and for packaging nascent virus particles. Thus, the SV40-influenza fusion RNA transcripts were probably rendered nonfunctional by the 5' and 3' terminal SV40 sequences. For these reasons gene rescue was attempted using RNA transcripts synthesized from influenza cDNA, because techniques are available for preparing transcripts that are nearly true copies of a given DNA template, whereas influenza transcripts produced in infected cells from an SV40-recombinant vector are invariably flanked by SV40 sequences. Full length NA gene cDNA inserts were subcloned into the Gemini plasmid vector, which contains promoter sequences for the RNA polymerases of phages SP6 and T7, in opposite transcriptional orientation, flanking the polylinker segment from phage M13. The polylinker has a unique Xba 1 site suitable for insertion of influenza A/WSN/33 neuraminidase (NA) cDNA. Prior to transcription the recombinant plasmid NA cDNA was linearized by cleavage at a unique site in the polylinker as close as possible to the NA cDNA cloning site and downstream from NA cDNA and the SP6 or T7 promoter. The linearized Gemini vector was then employed to transcribe RNAs whose polarity was determined by the promoter used to catalyze synthesis. Initially, two recombinants (pG1-8 and pG1-1) utilizing the SP6 promoter were used to transcribe plus (+) strand RNAs.

For the rescue experiments, the pG1-8 recombinant Gemini vector was re-engineered by conventional techniques to remove as much of the undesirable polylinker sequence as possible. Currently, (+) strand RNA transcribed from the linearized altered recombinant vector, pdG1-8, contains 16 bases at the 5' end and 9 bases at the 3' end that are not present in the NA gene sequence.

Attempts to rescue such RNA transcripts of influenza A WSN NA cDNA during infection with an influenza A virus bearing another NA gene were unsuccessful. Because we cannot rule out the possibility that even a few non-influenza sequences at the 5' and 3' ends of transcripts prevent recognition by viral polymerases of the infecting influenza A virus, efforts are being made to modify the pdG1-8 recombinant transcription vector to produce influenza RNA transcripts with fewer or no non-influenza sequences (Markoff, Lai, Chanock).

Complementation Of Influenza A Virus Mutants by A Cloned Influenza A Viral Gene Expressed In Stably Transformed Cells

Efforts to achieve persistent expression of influenza A virus cloned cDNA in cells permissive for virus infection were initiated because such transformed cells should be useful for investigation of the molecular biology of influenza A virus and for isolation of specific viral mutants through complementation by the expressed gene. In this manner, naturally occurring or laboratory engineered mutants containing viable deletion mutations could be isolated and evaluated for their level of attenuation. Initially, simian cells permissive for influenza A virus infection were stably transformed with a full length cloned influenza A nucleoprotein (NP) gene under the control of an inducible metallothionein promoter and linked to a dihydrofolate reductase gene to facilitate selection of transformed cells by methotrexate. The transformed cells which were selected with methotrexate synthesized an influenza A viral NP which was indistinguishable from the NP synthesized in virus-infected cells with respect to molecular weight and intracellular localization. It was estimated that transformed (CV1-NP) cells produced only 1% of the amount of NP synthesized in simian cells infected with influenza A virus. Nonetheless, when the transformed cells were infected with influenza virus mutants which synthesized temperature sensitive NP, protein expressed by the cloned gene was able to complement the synthesis of plus-strand and minus-strand viral RNA for some mutants and only plus-strand synthesis for other mutants. This indicated that the influenza A NP expressed in the transformed cells exhibited functional activity. Furthermore, under appropriate conditions CV1-NP cells complemented the replication of NP ts mutant viruses under restrictive conditions, i.e., high temperature. This complementation effect is currently being analysed to gain a better understanding of the biological functions of the viral NP and to develop a strategy for isolation of viable deletion mutants (Ryan, Lai, Chanock).

Dengue Viral Genome and Gene Products

Dengue viruses are members of the flavivirus family. This family contains many viruses which play an important role in human disease, for example, dengue fever, yellow fever (YF), and Japanese B encephalitis. With the exception of yellow fever virus vaccine, which has effectively controlled yellow fever, specific immunoprophylaxis is generally not available against diseases caused by flaviviruses. Although dengue does not constitute a major threat to public health in the U.S., dengue viruses continue to cause epidemics in many geographic areas, notably in East Asia, the South Pacific, Africa, the Caribbean, and Central and South America, where their mosquito vectors breed extensively. In these regions dengue viruses cause large epidemics involving hundreds of thousands or millions of illnesses in which debilitating disease predominates. It should be noted that Rio de Janeiro, Brazil is currently experiencing an unusually large dengue type 1 virus epidemic which has already involved several hundred thousand persons in its initial phase. In addition to

the classical clinical picture, initially described as "break bone fever" in 1790 by Benjamin Rush, dengue viruses also cause a hemorrhagic shock syndrome in infants and young children that has a very high mortality. In several regions in East Asia dengue hemorrhagic shock is one of the leading causes of death in infants and young children. Control of the dengue viruses is a major concern for residents in the epidemic areas as well as for individuals traveling into such areas. With ample justification the WHO has designated the dengue viruses as one of 5 high priority targets for accelerated development of virus vaccines.

Characterization of dengue virus strains from different epidemics from 1944 to the present indicates that there are 4 serologically distinct serotypes, and within each dengue serotype minor variations in the RNA genome have also been identified. Previous studies have shown that the dengue virus genome is a positive strand, unsegmented RNA of approximately 42S. This value corresponds to a length of approximately 11,000 nucleotides, although the exact size of the viral RNA had not been determined when we initiated our studies. Similar to other members of the flavivirus group, dengue genomic RNA contains a cap structure of m⁷Gp at its 5'-terminus, but poly A is not present at the 3'-terminus. Dengue virus resembles poliovirus, another positive strand RNA virus, in that its virion RNA is infectious when inoculated into a permissive cell culture. This indicates that dengue viral proteins are not required to initiate infection.

Genome Organization and Sequence: Structural Proteins

Recombinant DNA techniques were employed to investigate the molecular biology of dengue viruses with the intent of developing immunoprophylactic measures against these viruses which are epidemic in many geographic areas and which usually cause more morbidity than other flaviviruses. DNA sequences (approximately 11,000 nucleotides) representing the full-length genome of the Dominica strain of dengue virus type 4 were cloned in pBR322 in *E.coli*. Initially we determined the sequence of the first 2,429 nucleotides at the 5'-terminus which includes the coding region for the structural proteins. The virion structural proteins are encoded in one long open reading frame specifying a polyprotein precursor which is apparently proteolytically cleaved by a mechanism resembling that proposed for expression of structural proteins of other flaviviruses such as yellow fever (YF) and West Nile (WN) viruses. The N-terminus for each of the dengue virus structural proteins was tentatively assigned by homology alignment to the corresponding sequence of YF or WN virus. Homology alignment of the dengue type 4 virus polyprotein with the polyproteins of two other flaviviruses, yellow fever and West Nile viruses, indicated that the 5' end of the dengue viral RNA encoded three structural proteins designated capsid (C), pre-matrix (pre M) and envelope (E) in that order. Comparison of sequence homology of flavivirus structural proteins suggests that dengue virus is more closely related to WN virus than to YF virus or Murray Valley encephalitis (MVE) virus. In addition, analysis of the extreme 5'- and 3'-terminal nucleotides of the dengue virus genome revealed an usual arrangement of sequences that may be involved in transcription, replication and packaging of viral RNA (Zhao, Mackow, Lai, Chanock).

Genome Organization and Sequence: Nonstructural Proteins

Cloning and sequence analysis of the remainder of the dengue viral RNA indicated that the entire genome is 10,644 nucleotides in length and contains a single long open reading frame that encodes a polyprotein of 3386 amino acids.

The polyprotein is cleaved post-translationally into the full complement of viral proteins by a mechanism similar to that proposed for gene expression of other flaviviruses. The 3' terminal 7500 nucleotides encode seven nonstructural (NS) proteins, NS1, NS2a, NS2b, NS3, NS4a, NS4b, and NS5 in that order. These proteins are produced in infected cells but are not incorporated into virus particles. NS1 is the major NS glycoprotein produced during virus infection, while NS3 and NS5 contain polymerase-like amino acid sequences. Except for NS1, the function of dengue NS proteins is not known. NS1 is displayed on the surface of infected cells and appears to be an important target for the immune system. Dengue NS proteins are more highly related to WN or MVE virus NS proteins than to YF virus NS proteins. In the nonstructural protein region of the polyprotein there is considerable conservation of hydrophobicity among these flaviviruses and a consensus sequence prevails at many proteolytic cleavage sites suggesting a close functional relationship for corresponding NS proteins. In view of the recent finding that the YF NS1 induces resistance in mice to challenge with YF virus, it is likely that the analogous dengue NS1 protein may prove useful in immunoprophylaxis against dengue infection (Mackow, Lai, Zhao).

Expression Of Structural Proteins and Nonstructural Protein NS1 By A Vaccinia - Dengue Recombinant Virus

The initial goal in this project was to express both protective antigens of dengue virus, i.e., the E glycoprotein and the NS1 nonstructural glycoprotein, from cloned dengue cDNA using vaccinia virus as a vector. Because these and other dengue proteins are presumably proteolytically cleaved from their polyprotein precursor, the 5' region of the dengue genomic cDNA that codes for all the structural proteins, (C, (PreM) M, and E) as well as the first two downstream nonstructural proteins, NS1 and NS2a, was inserted into vaccinia virus. This region was excised from a full-length cDNA copy of the dengue genome at the two conveniently located BglII sites that flank these genes. This DNA fragment contains 4040 base pairs and its cohesive termini were used for insertion of the fragment into the vaccinia virus-plasmid coexpression vector pSC11 at a BamHI site which is located downstream of the P7.5 vaccinia promoter. Since there are two other BamHI sites in the vector, it was necessary to partially digest pSC11 with BamHI for insertion of the dengue cDNA fragment. In this construct the dengue cDNA insert is under the control of the P7.5 early-late vaccinia promoter, the vector contains the *E.coli* β -galactosidase gene under the control of a separate (P11) vaccinia promoter and both chimeric genes are flanked by vaccinia thymidine kinase (TK) sequences. After transformation of *E.coli* with the pSC11-dengue recombinant vector, plasmids from transformants were screened for the presence of the dengue cDNA insert in the correct orientation of transcription. In this manner, a recombinant bearing dengue cDNA in the correct orientation was identified. Cells infected with vaccinia virus were transfected with the recombinant and the TK sequences of the recombinant directed recombination of the chimeric genes into the TK locus of the vaccinia genome yielding viable vaccinia virus recombinants which could be identified colorimetrically by their β -galactosidase activity.

Initially, dengue viral antigens were detected by indirect immunfluorescence in CV-1 cells infected with the vaccinia-dengue recombinant. Specific identification was made using a dengue type 4 virus E glycoprotein monoclonal or a polyvalent dengue type 4 virus antiserum prepared in mice. Vaccinia-dengue recombinant infected cells were then labelled with

35 S-methionine and the cell lysate was processed for immunoprecipitation in order to determine which dengue-specific viral products were expressed. SDS-polyacrylamide electrophoresis indicated that the dengue E glycoprotein monoclonal antibody precipitated a protein of approximately 50-55 kilo daltons (kd) molecular weight which is the expected size of the E glycoprotein. Similarly, a dengue type 2 virus NS1 antiserum precipitated a 35-38 kd protein; this size is consistent with the molecular weight of the NS1 protein. These observations suggest that both glycoproteins were synthesized and specifically processed in a manner similar to that of authentic dengue proteins in virus infected cells. Presumably other dengue proteins encoded in the cloned DNA fragment were also expressed, i.e., capsid protein (C), membrane protein (M) and its precursor (PreM). Evidence supporting this view was provided by an experiment in which immunoprecipitation with polyvalent dengue type 4 virus antiserum detected a third labelled band of approximate m.w. 28 kd, which is equivalent in size to the PreM protein. These observations suggested that all the dengue viral structural proteins as well as the NS1 nonstructural protein were processed by proteolytic cleavage of the polyprotein in the absence of dengue viral functions provided by the 5 distal non-structural proteins, N2b through NS5. Currently, cotton rats are being immunized with the vaccinia-dengue recombinant. If these animals develop a high titer of serum neutralizing and cytolytic antibodies directed at the E and NS1 glycoproteins, respectively, we will initiate immunogenicity studies in monkeys. Several species of monkeys are permissive for dengue virus infection and hence these primates can be challenged following immunization in order to evaluate protective efficacy of the vaccinia-dengue recombinant (Zhao, Lai, Chanock).

Antigenic and Virulence Polymorphism Among Dengue Type 4 Strains

The dengue virus subgroup of the flavivirus family contains 4 distinct serotypes (type 1 to type 4). Intratypic variation has been detected within several serotypes by oligonucleotide fingerprint analysis and by virus neutralization. Dengue type 4 virus variants recovered from the Caribbean during 1981-1982 appear to be different from the dengue type 4 virus prototype strain (H241) isolated in the Phillipines in 1956 as well as from recent isolates from Southeast Asia. During the past two years, dengue type 4 virus was the most frequent cause of dengue hemorrhagic shock in Bangkok, in contrast to the earlier epidemic pattern in which dengue type 2 viruses were most often implicated. For these reasons it is important to define in molecular terms: (a) genetic stability and diversity of dengue type 4 viruses isolated over a 30 year interval and (b) the possible involvement of specific virus strains, i.e., "virulent viruses", in severe dengue disease. For the first phase of this investigation 2 dengue type 4 virus variants, the prototype strain H241 and strain 2123 isolated from a hemorrhagic shock patient, were chosen for cloning and DNA sequence analysis. Three specific oligonucleotide primers corresponding to the central region of the established dengue 4 (Dominica strain) sequence were tested for their ability to prime reverse-transcription of the viral genome. Each strain was effectively primed by at least one oligonucleotide and both dengue genomic RNAs yielded cDNA approximately 5,000 nucleotides in length. These cDNA products will be cloned by the procedure established earlier. Cloned DNA will then be analyzed to determine the sequence coding for the two protective antigens, i.e., the envelope glycoprotein and the NS1 nonstructural glycoprotein. Finally, sequences will be compared in an attempt to identify hypervariable regions in the E and NS1 glycoproteins (Zhang, Lai).

Attempts To Engineer The Genome Of Dengue Type 4 Virus

Studies are underway to determine if cloned dengue cDNA, or an RNA transcript derived from it, is infectious after introduction into permissive cells. Initially, clones representing the entire dengue genome were ligated to yield a cDNA containing the complete dengue sequence. Next, SV40 DNA sequences which represent strong signals for transcription were introduced into the plasmid vector in an attempt to increase transcription and hopefully, infectivity of cloned dengue cDNA. Also, because dengue viral RNA is infectious, efforts were initiated to produce dengue RNA transcripts from cloned cDNA and to introduce them directly into cultured cells. The versatile in vitro transcription system of SP6 or T7 phage is being utilized to produce the RNA transcripts. The full-length dengue DNA will be inserted into an appropriate transcription vector such as Gemini 3 at its unique Pst I site. The dengue DNA recombinant will then be linearized with Kpn I making it possible to obtain "run-off" transcripts which can be assayed for infectivity. If these manipulations are successful, site-specific mutagenesis will be performed to construct dengue virus mutants containing mutations in strategic regions essential for viral replication and other gene functions. Dengue virus mutants constructed in this manner may exhibit an altered phenotype such as temperature-sensitivity of virus replication or reduced virulence for man (Breuning, Lai).

ROTAVIRUSES

Major strides have been made during the past decade in elucidating the etiological agents of diarrhea of infants and young children. Rotaviruses have emerged as the single most important etiologic agents of severe diarrhea of infants and young children in both developed and developing countries. In developing areas of the world the impact of diarrheal diseases is staggering. It has been estimated that 3-5 billion cases of diarrhea and 5-10 million diarrhea-associated deaths occur each year in Asia, Africa and Latin America. Moreover, diarrhea ranks first in morbidity and mortality in these regions. The importance of rotaviruses in these regions as a cause of severe diarrhea has been demonstrated consistently. In a one year study of infants and children coming to a treatment center with severe diarrheal illness in Bangladesh, Black et al found that rotaviruses were the most frequently detected pathogens in patients less than 2 years of age; 46% were rotavirus positive. Thus, the need for an effective rotavirus vaccine is clear. The goal of such a vaccine is to prevent severe rotavirus diarrhea during the first 2 years of life when this disease is most serious. Studies in experimental animals indicate that intestinal immunity plays a major role in resistance to rotavirus disease and hence current efforts are aimed at developing a live attenuated vaccine for oral administration.

The most promising and most extensively evaluated method for rotavirus immunoprophylaxis is the "Jennerian" approach in which a related rotavirus from a foreign host is used as the immunizing agent. A recent efficacy trial by Vesikari of a bovine rotavirus strain (NCDV) in Finnish infants demonstrated a protection rate of over 80% against clinically significant diarrhea.

Rhesus Rotavirus Vaccine. As described in last years' annual report another animal rotavirus strain, rhesus rotavirus (RRV), is under intensive study in LID as a vaccine candidate. This simian rotavirus has not been recovered under natural conditions from man and is thus not a virus of the human heritage.

Although the genes of RRV exhibit significant divergence in sequence from the corresponding genes of human rotaviruses, this simian rotavirus is similar if not identical to human rotavirus type 3 when tested by neutralization. Also RRV grows efficiently in a semi-continuous, virus-free diploid cell strain (DBS FRhL-2) derived from fetal rhesus monkey lung. Its efficient growth in diploid cells constitutes an advantage because adventitious agents are frequently present in the usual primary cell culture systems used for vaccine production.

In last year's annual report we described phase 1 clinical trials (and one phase 2 study) in which the safety and antigenicity of various doses of rhesus rotavirus vaccine were evaluated in adults, and subsequently in children of progressively younger age. The RRV vaccine was found to be satisfactorily antigenic as well as non-reactogenic in adults and children. However, in several pediatric studies a febrile response and/or loose stools (albeit characteristically mild episodes) occurred when 6-12 month old infants were fed 10^3 PFU of virus. At a dose of 10^4 PFU the RRV retained its antigenicity in infants less than 6 months of age but did not cause significant reactions. Analysis of prevaccination serum RRV neutralizing antibody titers in several populations, some in which reactions occurred and some in which reactions did not occur, suggested that the reactions might be related to the absence of pre-existing serum antibody to RRV. Thus, prevaccination serum RRV antibodies may modify clinical reactions to RRV vaccine without significantly affecting infectivity or antigenicity. This experience prompted us to evaluate vaccination of a still younger age group in whom the potential protective effect of maternal antibodies might be greater. Because rotavirus diarrhea is an important problem in very early infancy in developing countries, it will probably be necessary to administer rotavirus vaccine during the first 2 months of life or perhaps even at birth. It has been estimated by Halsey and Galazka that 10% to 40% of infants in such countries are seen by a health care provider only at the time of birth. However, successful immunization during the neonatal period may be inhibited by very high levels of maternal antibodies acquired via the placenta or by breast feeding. For this reason we sought to identify a dose of RRV vaccine that was sufficiently infectious for young infants that it stimulated a silent immunizing infection under the cover of passively acquired, maternal RRV antibodies. A clinical trial involving 49 one to 4 month old infants who were fed 10^4 PFU of RRV vaccine was carried out in stepwise fashion in Venezuela. This dose proved to be nonreactogenic but antigenic; 75% of the infants developed a four fold or greater rise in antibody in response to vaccine (Kapikian, Flores, Glass, Midthun, Hoshino, Chanock).

Following the identification of a safe antigenic dose of vaccine several phase two double blind field trials were initiated with various collaborators in the U.S. and other parts of the world in order to assess the efficacy of this vaccine in infants under 6 months of age. The Epidemiology Section provided laboratory support for most of these studies. To date, the results are promising with regard to the absence of significant reactions in infants less than 6 months of age.

Reassortant Rotaviruses. If the "Jennerian" approach utilizing a simian rotavirus is not effective in inducing satisfactory protection against all human rotavirus serotypes, RRV could be used as a donor of attenuating genes that would be transferred to reassortant rotaviruses containing the major neutralization protein (VP7) of human rotavirus serotype 1, 2 or 4. Such

single gene substitution reassortants have been prepared for human rotavirus serotypes 1, 2, and 4. Each such reassortant has 10 RRV genes and a single human rotavirus gene, the one coding for the major neutralization antigen VP7. In addition, other reassortants with single gene substitutions, e.g. 10 genes from the UK bovine rotavirus plus the gene encoding VP7 for each of the 4 human serotypes, have been prepared (Midthun, Kapikian, Chanock).

Phase I trials of a DxRRV reassortant (VP7 of human rotavirus D, serotype type 1) and a DS-1xRRV reassortant (VP7 of human rotavirus DS-1, serotype 2) have just been completed at the Francis Scott Key Hospital of Johns Hopkins University in collaboration with Drs. Clements and Sears. Initially, each reassortant was administered by the oral route to 2 volunteers who had a high level of pre-existing serum neutralizing antibodies to the homologous reassortant. None of these 4 volunteers developed illness or shed rotavirus. The DxRRV reassortant was then fed to 8 volunteers with the lowest available homologous serum neutralizing antibody titer (1:160). Similarly, the DS-1xRRV reassortant was fed to 14 volunteers with the lowest available homologous serum neutralizing antibody titer (<1:80). None of these volunteers developed gastroenteric symptoms. The appropriate rotavirus was recovered from some of the volunteers in each group suggesting that these reassortants may exhibit satisfactory infectivity and antigenicity in fully susceptible infants. Phase I clinical trials of the reassortants are now underway in children (Kapikian, Midthun, Chanock).

Role of Fourth Gene In Virulence

Rotaviruses cause a wide spectrum of effects ranging from asymptomatic infection in neonates to severe dehydrating diarrheal illness in older infants and young children. Virulence is not related serotype because each of the four distinct human rotavirus serotypes has been associated with both silent and symptomatic infections.

Rotaviruses possess two outer capsid proteins, designated VP7 and VP3, which are considered to be independent neutralization antigens. VP7 which has a mol. wt. of 37K is encoded by genomic RNA segment 8 or 9, whereas, VP3 which has a mol. wt. of 84K is encoded by genomic RNA segment 4. VP3 is also responsible for restriction of growth of fastidious human rotaviruses in tissue culture and for hemagglutinating activity. Trypsin cleavage of VP3, which yields 2 polypeptides, VP8 and VP5, with molecular weights of 28,000 and 60,000, respectively, is required for the activation of infectivity of rotaviruses.

Initially, variation in rotavirus virulence was investigated by determining the extent of genetic relatedness among each of the corresponding genes of human rotaviruses isolated from: (a) infants or children with gastroenteritis and (b) asymptomatic newborn infants infected in the nursery. Genetic relationships were determined by RNA-RNA hybridization techniques.

³²P-labeled single stranded (ss) RNAs produced by in vitro transcription from viral cores of the different strains tested were used as probes in two different hybridization assays: a) undenatured genomic RNAs were resolved by polyacrylamide gel electrophoresis (PAGE), denatured in situ, electrophoretically transferred to DBM paper (Northern blots) and then hybridized to the probes under two different conditions of stringency; and b) denatured genomic double stranded (ds) RNAs were hybridized to the probes in

solution and the hybrids which formed were identified by PAGE and autoradiography.

When analyzed by Northern blot hybridization at a low level of stringency all the genes from the strains tested cross-hybridized providing evidence for some sequence homology in each of the corresponding genes. However, when hybridization stringency was increased, a difference in gene 4 sequence was detected between strains recovered from asymptomatic newborn infants ("nursery strains") and strains recovered from infants and young children with diarrhea. Although the nursery strains exhibited serotypic diversity, i.e., each of the four strains tested belonged to a different serotype, the fourth gene appeared to be highly conserved. Similarly, each of the virulent strains tested belonged to a different serotype, but nonetheless, there was significant conservation of sequence among the fourth genes of these viruses (Flores, Midthun, Hoshino, Kapikian, Chanock).

These results were confirmed and extended during experiments in which RNA/RNA hybridization was carried out in solution and the resulting hybrids analyzed by PAGE and autoradiography. Under these conditions, the fourth genes of the nursery strains were closely related to each other but not to the fourth genes of the virulent viruses. Full-length hybrids did not form between the fourth genes from the nursery strains and the corresponding genes from the strains recovered from symptomatic infants and young children. Conservation of the fourth gene among "nursery strains" of rotavirus and conservation of a different fourth gene among virulent rotaviruses occurs independent of serotype (VP7), subgroup specificity (VP6) or the remaining genes that code for other structural or nonstructural proteins (Flores, Midthun, Hoshino, Kapikian, Chanock).

Gene four sequence dimorphism was then investigated by sequence analysis in an attempt to elucidate the molecular basis of attenuation of the "nursery strains" that produce asymptomatic infection in newborns. The N-terminal region of the fourth gene which codes for VP8, the cleavage region and the N terminus of VP5 was sequenced from single stranded RNA transcripts prepared from viral cores of seven strains. These included four virulent human rotavirus strains each representing a different human rotavirus serotype (Wa, VA70, P and DS-1), four asymptomatic human rotavirus strains representing the same 4 human rotavirus serotypes (M37, 1076, McM and ST3) and a candidate vaccine simian rotavirus strain (MMU18006) (Gorziglia, Hoshino, Buckler-White, Kapikian, Chanock).

Sequence analysis indicated that the fourth gene plus (+) strand RNA has a 5' conserved nontranslated sequence of 9 nucleotides and encodes a VP8 protein of 240 amino acids in human rotavirus strains and 241 amino acids in simian rotavirus strains. Human and simian rotaviruses exhibit many similarities in this region of their genome, including identical N-terminal amino acid sequences, conservation of arginine at the two trypsin cleavage sites and the position of a cysteine residue. Alignment of amino acid sequences of the VP8 protein, the downstream cleavage region and the N-terminus of VP5 of asymptomatic and virulent human rotavirus strains indicates a high degree of homology (96% or more) among the asymptomatic viruses (serotypes 1, 2, 3 and 4), while homology between asymptomatic strains and virulent viruses is considerably less (68-72%). A high degree of conservation of amino acid sequence (92-97%) is also observed among 3 of the virulent strains (serotypes

1, 3 and 4). At 48 positions in the protein sequence of VP8, the cleavage region and the N terminus of VP5 an amino acid is conserved among asymptomatic rotaviruses, while a different amino acid is conserved among virulent rotaviruses. Notably, 3 of these differences are located within the short cleavage region which extends only 6 amino acids between VP8 and VP5. This suggests that a difference in optimal conditions for cleavage may be responsible in part for the observed difference in virulence. However, 3 or 4 conserved differences were also noted within a stretch of 6 amino acids in 6 other regions of VP8 or the N terminus of VP5. Altogether 45 amino acid positions were specifically conserved outside the cleavage region among asymptomatic rotaviruses, while a different amino acid was conserved at each of these sites among virulent rotaviruses. Some or all of these conserved sites outside the cleavage region may also play a role in the diminished virulence of the newborn nursery strains. At this time it is not possible to establish the relative importance of the various conserved sequences of the fourth gene product as determinants of virulence. Perhaps the critical sites are limited to the trypsin cleavage region. On the other hand, the determinants of virulence may be more widely distributed throughout the entire VP3 including the 3' region that was not analyzed in our study (Gorziglia, Hoshino, Buckler-White, Kapikian, Chanock).

These findings suggest that the fourth genes of virulent and asymptomatic human rotavirus strains represent two lines of divergent evolution from a common ancestor. Also, it is likely that the asymptomatic strains derived their fourth gene by reassortment rather than by sequential mutations.

Genetic Variation of Rotaviruses. Analysis of wild type rotavirus strains from the community suggest that gene reassortment occurs frequently. The study of nosocomial strains, on the other hand, suggests that the rotavirus genome undergoes sequential mutation, i.e., genetic drift, infrequently. Rotaviruses shed by newborn infants in the nursery of a large maternity hospital in Caracas, Venezuela were studied over a 4 year interval. Viral RNAs extracted from over 70 virus positive stool samples exhibited the same migration pattern during gel electrophoresis. Furthermore, by performing RNA-RNA cross-hybridization reactions, complete identity was observed between every pair of specimens tested. These observations on the genomic stability of the nursery strains contrast with the high degree of genetic variation seen among strains circulating concurrently in the community. This suggests that the high degree of genetic heterogeneity among rotaviruses in the community does not result primarily from accumulation of successive mutations; instead gene reassortment appears to be responsible (Green, Flores).

In order to study genetic stability in greater detail, sequence analysis was performed in an attempt to detect mutations which were not identified by the hybridization analysis. Ten strains obtained at different times from the same nursery over a 4 four year period were analyzed by sequencing 30% of the VP7 gene at its N-terminus. The middle region of the VP7 sequence was also analyzed. The VP7 gene was selected for analysis because it is under the greatest immunologic pressure and hence selection for mutations should be strongest. Alteration of sequence was not detected in any of the strains examined. This finding supports the view that the rotavirus genome does not undergo genetic drift with high frequency (Green, Flores).

solution and the hybrids which formed were identified by PAGE and autoradiography.

When analyzed by Northern blot hybridization at a low level of stringency all the genes from the strains tested cross-hybridized providing evidence for some sequence homology in each of the corresponding genes. However, when hybridization stringency was increased, a difference in gene 4 sequence was detected between strains recovered from asymptomatic newborn infants ("nursery strains") and strains recovered from infants and young children with diarrhea. Although the nursery strains exhibited serotypic diversity, i.e., each of the four strains tested belonged to a different serotype, the fourth gene appeared to be highly conserved. Similarly, each of the virulent strains tested belonged to a different serotype, but nonetheless, there was significant conservation of sequence among the fourth genes of these viruses (Flores, Midthun, Hoshino, Kapikian, Chanock).

These results were confirmed and extended during experiments in which RNA/RNA hybridization was carried out in solution and the resulting hybrids analyzed by PAGE and autoradiography. Under these conditions, the fourth genes of the nursery strains were closely related to each other but not to the fourth genes of the virulent viruses. Full-length hybrids did not form between the fourth genes from the nursery strains and the corresponding genes from the strains recovered from symptomatic infants and young children. Conservation of the fourth gene among "nursery strains" of rotavirus and conservation of a different fourth gene among virulent rotaviruses occurs independent of serotype (VP7), subgroup specificity (VP6) or the remaining genes that code for other structural or nonstructural proteins (Flores, Midthun, Hoshino, Kapikian, Chanock).

Gene four sequence dimorphism was then investigated by sequence analysis in an attempt to elucidate the molecular basis of attenuation of the "nursery strains" that produce asymptomatic infection in newborns. The N-terminal region of the fourth gene which codes for VP8, the cleavage region and the N terminus of VP5 was sequenced from single stranded RNA transcripts prepared from viral cores of seven strains. These included four virulent human rotavirus strains each representing a different human rotavirus serotype (Wa, VA70, P and DS-1), four asymptomatic human rotavirus strains representing the same 4 human rotavirus serotypes (M37, 1076, McM and ST3) and a candidate vaccine simian rotavirus strain (MMU18006) (Gorziglia, Hoshino, Buckler-White, Kapikian, Chanock).

Sequence analysis indicated that the fourth gene plus (+) strand RNA has a 5' conserved nontranslated sequence of 9 nucleotides and encodes a VP8 protein of 240 amino acids in human rotavirus strains and 241 amino acids in simian rotavirus strains. Human and simian rotaviruses exhibit many similarities in this region of their genome, including identical N-terminal amino acid sequences, conservation of arginine at the two trypsin cleavage sites and the position of a cysteine residue. Alignment of amino acid sequences of the VP8 protein, the downstream cleavage region and the N-terminus of VP5 of asymptomatic and virulent human rotavirus strains indicates a high degree of homology (96% or more) among the asymptomatic viruses (serotypes 1, 2, 3 and 4), while homology between asymptomatic strains and virulent viruses is considerably less (68-72%). A high degree of conservation of amino acid sequence (92-97%) is also observed among 3 of the virulent strains (serotypes

1, 3 and 4). At 48 positions in the protein sequence of VP8, the cleavage region and the N terminus of VP5 an amino acid is conserved among asymptomatic rotaviruses, while a different amino acid is conserved among virulent rotaviruses. Notably, 3 of these differences are located within the short cleavage region which extends only 6 amino acids between VP8 and VP5. This suggests that a difference in optimal conditions for cleavage may be responsible in part for the observed difference in virulence. However, 3 or 4 conserved differences were also noted within a stretch of 6 amino acids in 6 other regions of VP8 or the N terminus of VP5. Altogether 45 amino acid positions were specifically conserved outside the cleavage region among asymptomatic rotaviruses, while a different amino acid was conserved at each of these sites among virulent rotaviruses. Some or all of these conserved sites outside the cleavage region may also play a role in the diminished virulence of the newborn nursery strains. At this time it is not possible to establish the relative importance of the various conserved sequences of the fourth gene product as determinants of virulence. Perhaps the critical sites are limited to the trypsin cleavage region. On the other hand, the determinants of virulence may be more widely distributed throughout the entire VP3 including the 3' region that was not analyzed in our study (Gorziglia, Hoshino, Buckler-White, Kapikian, Chanock).

These findings suggest that the fourth genes of virulent and asymptomatic human rotavirus strains represent two lines of divergent evolution from a common ancestor. Also, it is likely that the asymptomatic strains derived their fourth gene by reassortment rather than by sequential mutations.

Genetic Variation of Rotaviruses. Analysis of wild type rotavirus strains from the community suggest that gene reassortment occurs frequently. The study of nosocomial strains, on the other hand, suggests that the rotavirus genome undergoes sequential mutation, i.e., genetic drift, infrequently. Rotaviruses shed by newborn infants in the nursery of a large maternity hospital in Caracas, Venezuela were studied over a 4 year interval. Viral RNAs extracted from over 70 virus positive stool samples exhibited the same migration pattern during gel electrophoresis. Furthermore, by performing RNA-RNA cross-hybridization reactions, complete identity was observed between every pair of specimens tested. These observations on the genomic stability of the nursery strains contrast with the high degree of genetic variation seen among strains circulating concurrently in the community. This suggests that the high degree of genetic heterogeneity among rotaviruses in the community does not result primarily from accumulation of successive mutations; instead gene reassortment appears to be responsible (Green, Flores).

In order to study genetic stability in greater detail, sequence analysis was performed in an attempt to detect mutations which were not identified by the hybridization analysis. Ten strains obtained at different times from the same nursery over a 4 four year period were analyzed by sequencing 30% of the VP7 gene at its N-terminus. The middle region of the VP7 sequence was also analyzed. The VP7 gene was selected for analysis because it is under the greatest immunologic pressure and hence selection for mutations should be strongest. Alteration of sequence was not detected in any of the strains examined. This finding supports the view that the rotavirus genome does not undergo genetic drift with high frequency (Green, Flores).

Identification of A Second Major Rotavirus Neutralization Antigen - VP3.

Antiserum prepared against the M37 strain of rotavirus, recovered from an asymptomatic neonate in Venezuela, neutralized two prototype human rotaviruses that define two separate serotypes - serotype 1 (Wa) and serotype 4 (ST3). This indicated that the M37 strain is a naturally occurring intertypic rotavirus. Genetic analysis of neutralization antigens established that the observed dual serotype specificity of M37 resulted from sharing the VP3 outer capsid protein with the ST3 virus and the VP7 outer capsid protein with the Wa virus. Thus, M37 exhibits the VP3 neutralization specificity of the ST3 virus and the VP7 neutralization specificity of the Wa virus. Analysis of single VP3 gene substitution reassortants indicated that VP3 was as potent an immunogen as VP7. These observations indicate that the serotype specificity of neutralizing antibodies elicited by VP3 can differ from the serotype specificity of neutralizing antibody elicited by VP7, indicating the need for a dual system of rotavirus classification in which the neutralization specificity of both VP3 and VP7 outer capsid proteins are identified (Hoshino, Kapikian).

Relative Importance of VP3 and VP7 Outer Capsid Proteins as Protective Rotavirus Antigens.

Porcine OSU and Gottfried rotaviruses are distinct by neutralization and thus both their VP3 and VP7 outer capsid proteins are unrelated antigenically. In order to study these neutralization proteins a single gene substitution OSU x Gottfried porcine rotavirus reassortant (11-1) was generated by gene reassortment. This reassortant derived 10 genes (including the fourth gene encoding VP3) from the OSU strain (serotype 5) and only the ninth gene (encoding the other major neutralization glycoprotein [VP7]) from the Gottfried strain (serotype 4). Gnotobiotic piglets fed this reassortant developed high levels of neutralizing antibodies not only to Gottfried, mediated by VP7, but also to OSU, mediated by VP3. This established that VP3 is as effective an immunogen as VP7 in inducing neutralizing antibodies during experimental intestinal infection. When a group of piglets initially infected with the reassortant was challenged three weeks later with virulent Gottfried virus, complete protection was observed as indicated by failure of symptoms to develop and failure to detect virus shedding. When another group of piglets previously infected with the reassortant was challenged with virulent OSU virus, complete protection against diarrhea was also observed and virus shedding was delayed in onset and decreased in duration. These observations indicate that: (i) VP3 antibodies induced during initial rotavirus infection confer resistance to disease produced by virulent rotavirus and (ii) a reassortant rotavirus bearing VP3 and VP7 neutralization antigens derived from separate rotaviruses which are distinct by neutralization induces immunity to both parental viruses (Hoshino, Kapikian).

Identification Of Serotype Using Single VP7 Gene Substitution Rotavirus

Reassortants. Single human rotavirus VP7 gene substitution reassortants were shown to be useful for studying the genetic relatedness of genes coding for the VP7 outer capsid protein of human rotaviruses belonging to serotypes 1, 2, 3 and 4. Double-stranded (ds) genomic RNAs of human rotaviruses belonging to serotype 1, 2, 3 or 4 were hybridized to single stranded (ss) mRNA probes derived from human-bovine rotavirus reassortants containing only the VP7 gene of their human rotavirus parent. Bovine rotavirus genes do not hybridize to the corresponding human rotavirus genes and thus hybridization can only occur between the human VP7 gene in the labeled probe and the VP7 gene present in the human rotavirus being tested. The specificity of this approach was established by the observation that VP7 genes of rotaviruses belonging to the same serotype

hybridized readily, whereas, hybridization was not detected between VP7 genes of rotaviruses belonging to different serotypes. Hybridization of viral RNAs using single human rotavirus VP7 gene substitution reassortants as probes may provide a rapid, efficient technique for identifying the VP7 serotype of rotaviruses derived from clinical or epidemiologic studies. The hybridization technique would circumvent the need for tissue culture adaption of isolates and subsequent serotype analysis by tissue culture neutralization (Midthun, Hoshino, Flores, Kapikian).

Molecular Mechanisms of Adenovirus Virulence.

Research was initiated to determine the adenovirus gene products required for cell damage and to establish the molecular mechanisms effecting the pathogenesis of adenovirus diseases. The cotton rat is an ideal animal model for these studies because the pathology of the pneumonia and upper respiratory disease produced by type 5 adenovirus in this animal is similar to that observed in humans. Basic experiments have been carried out to establish the parameters of the infection: kinetics of viral replication and clearance of virus from the lung; the temporal relationship of viral replication to the development of pneumonia; the cellular sites of viral replication in the lung; the immune response to infection; and the establishment of viral latency. Two species of cotton rats, Sigmodon hispidus and S. fulviventer, were compared in these studies; the S. hispidus proved to be the better species for investigating pathogenesis of pneumonia, but the S. fulviventer, appears to have distinct advantage for studies of the latent state. Currently a variety of deletion, insertion, and conditionally lethal mutants are being studied in an effort to determine the gene product or gene products responsible for cell damage and subsequent development of pneumonia (Ginsberg, Prince).

Adenovirus As A Vector For Expression Of Foreign Viral Genes.

An adenovirus expression vector, using the vaccine strain of type 4 adenovirus, is being constructed to develop an alternate viral vaccine containing the rotavirus gene encoding the major antigen that induces neutralizing antibodies. This vaccine would have a number of advantages for prevention of rotavirus infections throughout the world (Flores, Green, Ginsberg).

Honors and Awards

Robert M. Chanock

- Invited to deliver 1st annual Arthur K. Saz Microbiology-Immunology lecture, Georgetown University School of Medicine, October 9, 1985.
- Invited to deliver lecture at Symposium on Respiratory Syncytial Virus, Downstate Medical Center, State University of New York, Brooklyn, New York, October 16, 1985.
- 1985-86 Praxis Biologics Academic Visiting Professor of Pediatric Infectious Diseases In Department of Pediatrics, Duke University Medical Center, Dec. 16-18, 1985.
- Served on Scientific Review Committee of Research Institute of Scripps Clinic and Research Foundation, La Jolla, CA, Jan. 14-15, 1986.
- Invited to participate in Vaccines and Related Biologic Products Advisory Committee, Office of Biologics Research and Review, Food and Drug Admin., Bethesda, MD., Jan. 24, 1986.
- Co-organizer, Wallace P. Rowe Second Annual Symposium on Animal Virology, Bethesda, MD, Feb. 3-4, 1986.
- Member of 1986 Class Membership Committee, National Academy of Sciences, Feb. 8, 1986.
- Invited to participate in Vaccine Supply and Innovation Workshop, National Academy of Sciences, April 24-25, 1986.
- Invited to present lecture at International Symposium on Vaccine Development and Utilization, National Council for International Health, Agency for International Development, Pan American Health Organization, June 9-10, 1986.
- Member of Council of American Society of Virology, Santa Barbara, CA, Annual meeting June 22-26, 1986.
- Chairman, workshop: Viral vaccines, American Society of Virology, Santa Barbara, CA, June 23, 1986.
- Member of WHO Steering Committee on Acute Respiratory Viruses, Santa Barbara, CA, June 26-28, 1986.
- Co-organizer and co-chairman of Fourth Cold Spring Harbor Conference on Modern Approaches to New Vaccines Including Prevention of AIDS, Cold Spring Harbor, NY, September 9-14, 1986.
- Co-editor of Vaccines 86: New approaches to immunization. Developing vaccines against parasitic, bacterial, and viral diseases. Third Cold Spring Harbor Conference on Modern Approaches to Vaccines, Cold Spring Harbor, NY. Published by Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1986.

Albert Kapikian

Member, Steering Committee of the Scientific Working Group on Viral Diarrheas of the Diarrhoeal Diseases Control Program of WHO.

Invited as temporary adviser to attend 11th meeting of Steering Committee of Scientific Working Group on Viral Diarrheas of Diarrheal Diseases Control Program of WHO, Geneva, Switzerland, Aug. 20-23, 1985.

Member, Viral Disease Panel of US-Japan Cooperative Medical Science Program

Co-chairman of session on Viral Gastroenteritis at Nineteenth Annual Working Conference on Dengue and Other Arboviruses, Viral Gastroenteritis and Rabies, NIH, Bethesda, MD and Ft. Detrick, MD, October 28-30, 1985.

Invited participant Diatech-PATH workshop on Diarrheal Diseases, Washington, D.C., Dec. 5, 1985.

Invited to write "Perspective" article for Journal of Infectious Diseases.

Invited to speak at Intramural Research Seminar, Deputy Director for Science, Emeritus, Dr. Stetten, Bethesda, MD, Jan. 17, 1986.

Invited to be member of WHO Scientific Working Group on Immunology, Microbiology & Vaccine Development 1986-1989.

Invited to 1st meeting of the Scientific Working Group on Immunology, Microbiology and Vaccine Development, Geneva, Switzerland, Mar. 4-7, 1986.

Invited speaker at Seminar "Human Gastroenteritis Viruses in the Environment" at Annual meeting of American Society for Microbiology, Washington, D.C., March 23-28, 1986.

Invited speaker PHS-AID US-India Vaccine Action Program-Vaccine Development Opportunities meeting, Bethesda, MD, March 31-April 2, 1986.

Participant, Meeting of Collaborating Rhinovirus Investigators to extend rhinovirus numbering system, Columbus, Ohio, June 5, 1986.

Invited speaker at "International Symposium on Vaccine Development and Utilization, Washington, D.C., June 9-10, 1986.

Membership Committee, American Epidemiological Society.

Participant, American Society of Virology, Santa Barbara, CA, June 21-23, 1986.

Invited speaker, Cold Spring Harbor Symposium on Vaccines, Cold Spring Harbor, NY, September 9-14, 1986.

Ching-Juh Lai

Invited speaker, Nineteenth Annual Working Conference on Dengue and Other Arboviruses, Viral Gastroenteritis and Rabies, US-Japan Cooperative Medical Science Program, NIH, Bethesda, MD, October 28-30, 1985.

Workshop convenor for The Togavirus Workshop, American Society of Virology, Santa Barbara, CA, June 21-23, 1986.

Invited speaker, First SCBA International Symposium and Workshop, San Francisco, CA, June 30-July 2, 1986

Brian Murphy

Invited speaker, The Biology of Negative Strand Viruses, Cambridge, September 15-20, 1985.

Invited speaker on immune response of infants & children and animals to infection or immunization with RSV, Praxis Biologics, March 23, 1986.

Convenor, American Society of Virology, Session on Measles and Mumps Viruses, Santa Barbara, CA, June 21-23, 1986.

Consultant, WHO Scientific Conference on Paramyxoviruses, WHO Program for Vaccine Development, Acute Respiratory Viruses, Santa Barbara, CA, June 24-25, 1986.

Invited to participate, WHO Consultation on Molecular Epidemiology of Influenza Viruses in Athens, Georgia, September 5-6, 1986.

Invited speaker, Cold Spring Harbor Symposium on Vaccines, Cold Spring Harbor, NY, September 9-14, 1986.

Robert H. Purcell

Invited participant, WHO Scientific Group on Non-A, Non-B Hepatitis, Delta Antigen-Associated Hepatitis and Blood-Borne Human Retroviruses, Tokyo, Japan, October 4-6, 1985.

Invited speaker, Shanghai International Symposium on Liver Cancer and Hepatitis, Shanghai, Republic of China, January 15-17, 1986.

Recipient, Distinguished Scientist Award of the Society for Experimental Biology and Medicine, Washington, D.C. Chapter, February 19, 1986.

Invited participant, Annual Meeting, U.S.-Japan Bilateral Science Agreement, Viral Hepatitis Panel, Williamsburg, Virginia, March 7-8, 1986.

Invited speaker, Annual Meeting of the American Society for Microbiology, Washington, D.C., March 23-28, 1986.

Invited participant, U.S.-India Vaccine Action Program, Vaccine Development Priorities Meeting, Bethesda, Maryland, April 1-5, 1986.

Invited speaker, AIDS inoculum for chimpanzees, San Antonio, Texas, April 4-5, 1986

Invited speaker, UCLA Symposia on Positive Strand RNA Viruses, Keystone, Colorado, April 20-26, 1986.

Invited speaker, International Symposium: The Hepatitis Delta Virus (HDV) and its Infection, Saint Vincent, Italy, June 19-20, 1986.

Invited participant, Steering Committee of the WHO Programme on the Development of Vaccines for Hepatitis A and Poliomyelitis, Hampstead, England, June 30-July 1, 1986.

Invited speaker, Cold Spring Harbor Symposium on Vaccines, Cold Spring Harbor, NY, September 9-14, 1986.

Kathleen Coelingh

Invited speaker, The Biology of Negative Strand Viruses, Cambridge, September 15-20, 1985.

Invited speaker, American Society of Virology, Santa Barbara, CA, June 21-23, 1986.

Consultant, WHO Scientific Conference on Paramyxoviruses, WHO Program for Vaccine Development, Acute Respiratory Viruses, Santa Barbara, CA, June 24-25, 1986.

Invited speaker, Cold Spring Harbor Symposium on Vaccines, Cold Spring Harbor, NY, September 9-14, 1986.

Peter Collins

Consultant, WHO Scientific Conference on Paramyxoviruses, WHO Program for Vaccine Development, Acute Respiratory Viruses, Santa Barbara, CA, June 24-25, 1986.

Invited speaker, American Society of Virology, Santa Barbara, CA, June 21-23, 1986.

Invited speaker, Cold Spring Harbor Symposium on Vaccines, Cold Spring Harbor, NY, September 9-14, 1986.

Jorge Flores

Invited speaker, Double-Stranded RNA Viruses, Oxford, England, September 9-13, 1986.

Invited speaker, Nineteenth Annual Working Conference on Dengue and Other

Arboviruses, Viral Gastroenteritis and Rabies, NIH, Bethesda, MD, October 28-30, 1985.

Mario Gorziglia

Invited speaker, Cold Spring Harbor Symposium on Vaccines, Cold Spring Harbor, NY, September 9-14, 1986.

Yasutaka Hoshino

Invited speaker, Nineteenth Annual Working Conference on Dengue and Other Arboviruses, Viral Gastroenteritis and Rabies, NIH, Bethesda, MD, October 28-30, 1985.

Philip Johnson

Invited speaker, 1986 Annual meeting of the Infectious Diseases Society of America, New Orleans, LA, October 2-3, 1986.

Ruth Karron

Invited speaker, 1986 Annual meeting of the Infectious Diseases Society of America, New Orleans, LA, October 2-3, 1986.

Karen Midthun

Invited speaker, Nineteenth Annual Working Conference on Dengue and Other Arboviruses, Viral Gastroenteritis and Rabies, NIH, Bethesda, MD, October 28-30, 1985.

Robert Olmsted

Invited speaker, American Society of Virology, Santa Barbara, CA, June 21-23, 1986.

Invited speaker, Cold Spring Harbor Symposium on Vaccines, Cold Spring Harbor, NY, September 9-14, 1986.

Gregory Prince

Invited speaker on immunopotential on cotton rats, Praxis Biologics, March 23, 1986.

Invited speaker, American Society of Virology, Session on Measles and Mumps Viruses, Santa Barbara, CA, June 21-23, 1986.

Consultant, WHO Scientific Conference on Paramyxoviruses, WHO Program for Vaccine Development, Acute Respiratory Viruses, Santa Barbara, CA, June 24-25, 1986.

Invited speaker, Cold Spring Harbor Symposium on Vaccines, Cold Spring Harbor, NY, September 9-14, 1986.

Mark Snyder

Invited speaker, American Society of Virology, Santa Barbara, CA, June 21-23, 1986.

Melanie Spriggs

Invited speaker, American Society of Virology, Santa Barbara, CA, June 21-23, 1986.

John Ticehurst

Invited speaker, U.S.-Japan Cooperative Medical Science Program. Seventh Joint Conference on Hepatitis. Williamsburg, Virginia, March 7-8, 1986.

Invited speaker, Fifth International Conference on Comparative Virology. Alberta, Canada, May 4-10, 1986.

Invited speaker, IXth International Congress of Infectious and Parasitic Diseases. Munich, West Germany, July 20-26, 1986.

Bangti Zhao

Invited speaker, Cold Spring Harbor Symposium on Vaccines, Cold Spring Harbor, NY, September 9-14, 1986.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00308-05 LID
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>In Vitro</u> and <u>In Vivo</u> Studies of Hepatitis A Virus		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <div style="display: flex; justify-content: space-between; margin-top: 10px;"> <div> PI: Ruth A. Karron, M.D. Others: Stephen M. Feinstone, M.D. Robert H. Purcell, M.D. </div> <div> Guest Researcher, HV Section Medical Officer Head, HV Section </div> <div> LID, NIAID LID, NIAID LID, NIAID </div> </div>		
COOPERATING UNITS (if any) Smith-Kline-RIT (Dr. d'Hondt); Office of Biologics, FDA (Dr. Daemer)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Hepatitis Viruses Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 1.1	PROFESSIONAL: 0.9	OTHER: 0.2
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between; margin-top: 5px;"> <div> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input type="checkbox"/> (b) Human tissues </div> <div> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unraduated type. Do not exceed the space provided.) <p>Hepatitis A virus has been successfully adapted to growth in African green monkey kidney tissue culture. The virus is predominantly cell-associated and does not produce cytopathic effects (CPE). It was attenuated for chimpanzees after 10 or 20 serial tissue culture passages. The attenuated virus has been shown not to revert to virulence during serial passage through chimpanzees. The tissue culture-adapted HAV has been thrice cloned and clones representing passages 20 and 30 have been characterized in chimpanzees and marmosets and compared with wild-type virus. Immunization of chimpanzees with the attenuated virus protected most but not all vaccinated animals.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00309-05 LID
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Structural and Antigenic Analysis of Hepatitis A Virus		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Stephen M. Feinstone, M.D.	Medical Officer LID, NIAID
Others:	Robert H. Purcell, M.D.	Head, HV Sect. LID, NIAID
	John Ticehurst, M.D.	Senior Staff Fellow LID, NIAID
COOPERATING UNITS (if any) NIAID, LIG (Dr. Maloy); Fairfield Hospital, Fairfield, Victoria, Australia (Dr. I. Gust, Dr. A. Coulepis); Scripps Inst., La Jolla, California (Dr. R. Lerner)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Hepatitis Viruses Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS:	0.8	PROFESSIONAL: 0.4
		OTHER: 0.4
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.) Antibodies to hepatitis A virus structural proteins have been produced in guinea pigs by inoculating either SDS disrupted purified hepatitis A virus or in rabbits by inoculating synthetic peptides. These antibodies have been used to study the structural proteins of hepatitis A virus by western blot and the antigens of the virus by <u>in vitro</u> neutralization and radioimmunoassays. Preliminary data indicate that VPO of HAV may not be cleaved into VP2 and VP4.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00310-04 LID TERMINATED
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (40 characters or less. Title must fit on one line between the borders.) Molecular Biology of Hepatocellular Carcinoma		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Stephen M. Feinstone, M.D. Medical Officer LID, NIAID Others: Robert H. Purcell, M.D. Head, HV Sect. LID, NIAID		
COOPERATING UNITS (if any) NIAID, LIG (Dr. Maloy)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Hepatitis Viruses Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 0	PROFESSIONAL: 0	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p style="text-align: center;"> Production and characterization of monoclonal antibodies to hepatitis A virus are in progress. The development of new serologic tests for detection of monoclonal anti-HAV has simplified the procedure. Five specific monoclonal antibodies have been produced and are being analyzed. </p> <p style="text-align: center; margin-top: 100px;"> MERGED WITH Z01 AI 00309-05 LID TERMINATED 1986 </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00311-05 LID
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Search for New Hepatitis Agents		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	John Ticehurst, M.D.	Senior Staff Fellow LID, NIAID
Others:	Robert H. Purcell, M.D. Albert Kapikian, M.D.	Head, HV Section LID, NIAID Head, Epidemiology Sect. LID, NIAID
COOPERATING UNITS (if any) CC Dept Transfusion Med, NIH (Dr. Alter); Nat Inst Virol, Pune, India (Dr. Pavri); Med College, Srinagar, India (Dr. Khuroo); Mt Sinai Hosp, NYC (Dr. Popper); Inst Pasteur d'Algerie, Algiers, (Dr. Belabbes); Ivanovsky Inst., Moscow, USSR (Dr. Zhadonov), Walter Reed Army Inst of Res (Dr. Sjogren)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Hepatitis Viruses Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS:	1.3	PROFESSIONAL: 0.4 OTHER: 0.9
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) <p> New hepatitis agents continue to be recognized. Recently, a form of epidemic hepatitis occurring in India was found not to be caused by any of the recognized hepatitis viruses. Previous attempts to transmit an agent from acute-phase clinical samples to primates were partially successful. Transient low-level liver enzyme elevations and histopathologic changes consistent with hepatitis in liver biopsies were observed in some animals, but this was not uniform, and attempts to serially transmit an agent in chimpanzees and marmosets also produced irregular results. Efforts to identify viral agent(s) in recently obtained clinical specimens from India, Algeria and the USSR include primate transmission studies, immune electron microscopy, and cloned cDNA hybridization. Although an etiologic agent has not been identified in experimental animals and none of the specimens has hybridized with probes that detect a wide range of picornaviruses including HAV, several different virus-like particles have been visualized. Attempts to define a serologic relationship between hepatitis and one or more of these particles are in progress. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00312-05 LID
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Clinical and Experimental Studies of Hepatitis B Vaccines		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: John Ticehurst, M.D. Senior Staff Fellow LID, NIAID Others: Robert H. Purcell, M.D. Head, HV Sect. LID, NIAID		
COOPERATING UNITS (if any) Baylor School Med., Houston, TX (Dr. Hollinger); Div. Mol. Virol. & Immunol., G.U., Wash. DC (Dr. Gerin); Dept. Med., Rutgers Med. School, New Brunswick, NJ (Dr. McAuliffe); CDC, Atlanta, GA (Dr. Francis); Dept. Epid., Shanghai 1st Med. Coll. (Dr. Xu).		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Hepatitis Viruses Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 0.3	PROFESSIONAL: 0.2	OTHER: 0.1
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p style="margin-top: 10px;">Clinical testing and characterization of one lot of plasma-derived hepatitis B vaccine prepared by the NIAID have been completed. The vaccine was highly immunogenic, safe and well tolerated when tested in healthy persons ranging from infants to adults. Results from an efficacy trial in Asia indicate that the vaccine effectively prevents transmission of hepatitis B virus infection from mothers to infants. The sera from these infants are being analyzed for responses to specific HBsAg epitopes in an attempt to identify those that are most important.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00313-04 LID TERMINATED
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Molecular Biology of Hepatocellular Carcinoma		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Robert H. Purcell, M.D.	Head, HV Section LID, NIAID
Others:	Kendo Kiyosawa, M.D.	Visiting Associate LID, NIAID
	He Li-Fang, M.D.	Visiting Fellow LID, NIAID
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Hepatitis Viruses Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS:	0.8	PROFESSIONAL: 0.7 OTHER: 0.1
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> Many tumor-bearing animals develop antibodies to unique antigens associated with the oncogenic virus causing the tumor. These antigens, called "neoantigens," have been found in tumors caused by papovaviruses, adenoviruses, and herpes viruses. Hepatitis B virus, a hepadnavirus with suspected oncogenic potential, cannot be transmitted to non-primates but patients with HBV-associated hepatoma might be expected to have antibody to a HBV-associated neoantigen if one exists. Using a hepatoma cell line that contains integrated HBV DNA, we sought immuno-fluorescent antibody in sera of hepatoma patients. Approximately seven percent of sera from HBsAg-positive hepatoma patients contained an antibody that reacted with a nuclear antigen in the hepatoma cell line. This antigen was found in another hepatoma cell line that also contained integrated HBV genome but not in two other hepatoma cell lines lacking HBV genome. The antigen ("hepatitis B virus-associated nuclear antigen": HBNA) is being further characterized to determine if it is the product of a transforming gene. A different nuclear antigen was found in a human hepatoma cell line that did not contain HBV DNA. It was identified with serum from a patient with HBsAg negative hepatocellular carcinoma. The new antigen has characteristics similar to those of HBNA. </p> <p style="text-align: center; margin-top: 100px;">TERMINATED 1986</p> <p style="text-align: center; margin-top: 20px;">8-36</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00314-05 LID
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Woodchuck Virus		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Roger Miller, Ph.D. Senior Staff Fellow, HV Section LID, NIAID		
COOPERATING UNITS (if any) Div. Molec. Virol. & Immunol., Georgetown U, Washington, DC (Dr. Gerin); New York State College of Veterinary Medicine (Dr. B. Tennant)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Hepatitis Viruses Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda Maryland 20892		
TOTAL MAN-YEARS: <div style="text-align: center;">1.6</div>	PROFESSIONAL: <div style="text-align: center;">1.0</div>	OTHER: <div style="text-align: center;">0.6</div>
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The woodchuck hepatitis virus (WHV) is taxonomically and serologically related to the hepatitis B virus (HBV). Infection with each of these viruses is associated with acute and chronic hepatitis and hepatic cell carcinoma in their respective hosts; these associations appear to be etiological in nature. The WHV/woodchuck model system provides a convenient means of studying the relationship between virus and host in the oncogenic process. Tests specific for the WHV antigen-antibody systems have been developed. Genetic relationships between the hepadnaviruses and retroviruses suggest a common origin. Genetically altered WHV genomes, constructed by recombinant DNA techniques are being studied in woodchucks in order to determine the biological significance of individual gene products.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00317-05 LID
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Biology of Non-A, Non-B Hepatitis Agents		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Stephen M. Feinstone, M.D.	Medical Officer LID, NIAID
Others:	Robert H. Purcell, M.D.	Head, HV Section LID, NIAID
COOPERATING UNITS (if any) Blood Bank, NIH Clinical Center, Bethesda, MD (Dr. Alter, Dr. Shih, Dr. Esteban); CH, LMG, NIH, Bethesda, MD (Dr. Dawid, Dr. Sargent). Nihon U. School Med., Tokyo, Japan (Dr. Y. Shimizu)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Hepatitis Viruses Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.8	0.4	0.4
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The agent of non-A, non-B hepatitis continues to resist identification. A selected cDNA library was produced and screened for specific non-A, non-B hepatitis virus or disease related sequences, but none were identified. A second more highly selected library is presently being constructed. A cDNA expression library in λ gt 11 was constructed and screened with Dr. Shimizu's monoclonal antibody. No clones producing an antigen that reacts with the Shimizu antibodies have yet been isolated. Work is continuing on this library with Dr. Shimizu's and other antibodies.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00319-05 LID
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) New Approaches to the <u>In Vitro</u> Propagation of Non-Cultivable Agents		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: Robert H. Purcell, M.D.	Head, HV Section	LID, NIAID
COOPERATING UNITS (if any) Office of Biologics, FDA (Dr. Daemer)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Hepatitis Viruses Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 0.3	PROFESSIONAL: 0.1	OTHER: 0.2
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) <p> There is a need for an <u>in vitro</u> substrate for the cultivation of hepatitis viruses. Hepatocytes would seem a logical choice, but it is very difficult to obtain and maintain primate hepatocytes in culture. We are attempting to develop hepatocyte-hepatoma hybridomas of primate origin. Such hybrid cells would be expected to have the receptor sites and metabolic systems suitable for synthesis of hepatitis viruses and the ability of hepatoma cells to multiply indefinitely <u>in vitro</u>. Methods for the selection of hybrid cells without drug markers (i.e. use of vital dyes) have been developed. These and other vital dyes are being studied for their ability to detect metabolic changes in cells that are infected with viruses. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00321-05 LID
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Development, Characterization, and Use of Cloned Hepatitis A Virus (HAV) cDNA		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: John Ticehurst, M.D. Others: Stephen M. Feinstone, M.D. Robert H. Purcell, M.D. Jeffrey I. Cohen, M.D. Manfred Weitz, Ph.D. Ruth A. Karron, M.D.	Senior Staff Fellow Medical Officer Head, HV Sect. Medical Staff Fellow Fogarty Visiting Fellow Guest Worker	LID, NIAID LID, NIAID LID, NIAID LID, NIAID LID, NIAID LID, NIAID
COOPERATING UNITS (if any) Georgetown U (Baroudy): Columbia U, NY (Racaniello); Chiron Corp, CA (Dina); Germ Primate Ctr, FRG (Tracy); U Wisc (Palmenberg); U Turku, Finland (Hyypia); Genentech Corp, CA (Kleid); Scripps, CA (Lerner); LMB, NCI (Maizel); LIG, NIAID (Maloy); Athens Sch. Hygiene, Greece (Tassopoulos);		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Hepatitis Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 2.4	PROFESSIONAL: 1.3	OTHER: 1.1
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Double-stranded cDNA fragments were synthesized from wild type HAV strain HM-175 RNA. Cloned cDNA was used as a probe for detecting HAV RNA in tissue culture, serum, and fecal specimens by hybridization. Hybridization experiments also demonstrated that probes taken from any region of the HAV genome will not hybridize to RNA or cloned cDNA from a variety of other picornaviruses. In addition, from analysis of the complete nucleotide and predicted amino acid sequences of this genome and comparison with sequences from other picornaviruses, we have concluded that HAV has typical picornaviral genome organization but widely divergent sequences and it should be classified separately from other picornaviral genera. HAV VPg was demonstrated by the interaction between antibodies directed against a synthetic peptide, representing the VPg amino acid sequence predicted from cloned cDNA, and a covalent HAV RNA-protein (VPg) complex. The baculovirus expression system is being used in attempts to express the structural and nonstructural proteins of HAV.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00370-04 LID
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Studies of Acquired Immune Deficiency Syndrome (AIDS)		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Robert H. Purcell, M.D. Head, HV Section LID, NIAID		
COOPERATING UNITS (if any) NINCDS, NIH, Bethesda, MD (Dr. London); NCI, NIH (Dr. Gallo); New York Blood Center, NYC, NY (Dr. Stevens, Dr. Baker); Memorial Sloan Kettering Inst., NYC, NY (Dr. Gold); LIG, NIAID, NIH, Bethesda, MD (Dr. Folks, Dr. Fauci); Georgetown U. (Dr. Gerin).		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Hepatitis Viruses Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS:	0.2	PROFESSIONAL: 0.1 OTHER: 0.1
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>A new medical syndrome, Acquired Immune Deficiency Syndrome (AIDS) has recently been recognized. It is characterized by profound progressive depression of the immune system, resulting in repeated opportunistic infections and at least one type of neoplasm.</p> <p>The syndrome is usually if not always fatal.</p> <p>A retrovirus has been identified as the etiologic agent of AIDS. Attempts to develop a suitable primate animal model are in progress.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00404-03 LID
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Second and Third-Generation Hepatitis B Vaccines		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Robert H. Purcell, M.D. Head, HV Section LID, NIAID		
COOPERATING UNITS (if any) Georgetown University, Washington, DC (Dr. Gerin); Scripps Institute, La Jolla, California (Dr. Lerner); LBV, NIAID, NIH, Bethesda, MD (Dr. B. Moss)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Hepatitis Viruses Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 0.1	PROFESSIONAL: 0.1	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unraduated type. Do not exceed the space provided.) <p>Expense, availability and/or incomplete acceptance based upon an unfounded fear of infection with the agents of AIDS limit the impact of plasma-derived hepatitis B vaccines in developed and developing countries. There is therefore a need for new approaches to vaccine development. Recombinant DNA and synthetic peptide technologies appear to offer the best opportunities for the next generation of hepatitis B vaccines. Partial or complete protection against hepatitis B has been demonstrated in chimpanzees following vaccination with (a) recombinant derived subunit vaccine prepared in eukaryotic cells, (b) live recombinant vaccinia virus containing HBV genes, and (c) synthetic peptides representing HBsAg sequences. Attempts to identify the antigenic domains most important in stimulating neutralizing antibody are currently in progress.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00405-03 LID
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Inactivation of Hepatitis Viruses in Pooled Plasma Derivatives		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Robert H. Purcell, M.D. Head, HV Section LID, NIAID		
COOPERATING UNITS (if any) Georgetown U., Washington DC (Dr. Gerin); Southwest Fndt., San Antonio, TX (Dr. Eichberg); Revlon Inc., Tuckahoe, NY (Dr. Landaburu)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Hepatitis Viruses Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 0.7	PROFESSIONAL: 0.1	OTHER: 0.6
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p style="margin-top: 10px;"> The transmission of viral hepatitis by plasma derivatives continues to be an important medical problem that has been made more urgent by the similar epidemiology of the agent of acquired immune deficiency syndrome. Methods of inactivating viruses in plasma derivatives while retaining the biological potency of the products are being explored. These include the extraction of lyophilized or aqueous preparations with lipid solvents such as chloroform. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE		PROJECT NUMBER	
NOTICE OF INTRAMURAL RESEARCH PROJECT		Z01 AI 00406-03 LID	
PERIOD COVERED October 1, 1985 to September 30, 1986			
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Molecular Structure of HAV: Genomic Sequence and Organization			
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)			
PI: Jeffrey I. Cohen, M.D.		LID, NIAID	
Others: John R. Ticehurst, M.D.		Medical Staff Fellow	LID, NIAID
Stephen Feinstone, M.D.		Medical Officer	LID, NIAID
Manfred Weitz, Ph.D.		Visiting Fellow	LID, NIAID
Robert H. Purcell, M.D.		Head, HB Section	LID, NIAID
COOPERATING UNITS (if any) Lab. Mol. Genetics, Natl. Inst. of Child Health and Human Develop., NIH, Bethesda, MD (Dr. Jacob V. Maizel); Lab. Immunogen., NIAID, NIH, Bethesda, MD. (Dr. L. Walter Maloy); DMVI, Georgetown U. Sch. Med. (Dr. Baroudy)			
LAB/BRANCH Laboratory of Infectious Diseases			
SECTION Hepatitis Viruses Section			
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892			
TOTAL MAN-YEARS: 1.7		PROFESSIONAL: 1.0	OTHER: 0.7
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews			
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)			
<p>The complete nucleotide sequence of wild-type HAV strain HM-175 was determined. The genome is 7478 nucleotides long, followed by a poly A tail, and encodes a polyprotein of 2227 amino acids. On the basis of amino acid homology with other picornaviruses, putative cleavage sites for the polyprotein have been identified. The molecular weights of VP1, VP2, and VP3 predicted from the sequence data agree with those determined by biophysical methods. Proteins 2C and 3D are most homologous with other picornaviruses. HAV shares the putative active enzyme sites on proteins 3C and 3D with other picornaviruses. Comparison of wild-type HM-175 with a cell culture adapted strain (Najarian, PNAS 1985) shows 624 nucleotide differences (91.7% identity), but only 34 amino acid differences (98.5% identity).</p> <p>Wild-type HAV HM-175 has been passaged 30 times in primary African green monkey kidney (AGMK) cells. The resultant virus, P30 HM-175, is attenuated for chimpanzees. cDNA clones have been prepared from this latter virus and we are currently characterizing the clones. Comparison of the sequence of P30 with wild-type HM-175 HAV should help to locate regions of the genome responsible for adaptation to cell culture and attenuation.</p>			
8-45			

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00440-02 LID
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Construction of Full Length Hepatitis A Virus cDNA for Transfection		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Jeffrey I. Cohen, M.D.	Medical Staff Fellow LID, NIAID
Others:	John R. Ticehurst, M.D.	Medical Staff Fellow LID, NIAID
	Stephen M. Feinstone, M.D.	Medical Officer LID, NIAID
	Robert H. Purcell, M.D.	Head, HV Section LID, NIAID
COOPERATING UNITS (if any) Columbia University, NY (Dr. Racaniello); University of Alabama, Birmingham, Ala. (Dr. C. Morrow); U. Reading, UK (Dr. G. Almond).		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Hepatitis Viruses Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS:	0.6	PROFESSIONAL: 0.5 OTHER: 0.1
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>cDNA from hepatitis A virus (HAV) has been cloned into pBR322. Six cDNA clones which together span the entire genome were isolated and ligated together to form a single clone thought to represent full length HAV cDNA. Transfection of both tissue culture cells (<u>in vitro</u>) and marmosets (<u>in vivo</u>) with these plasmids failed to generate HAV. Fine structure mapping of the HAV cDNA indicated that about 40 base pairs had been deleted during the ligation process. The deletion was repaired, but transfection of marmosets and tissue culture cells still failed to generate HAV. An additional modified construct, differing by two nucleotides thought to be important for infectivity, also failed to generate HAV in marmosets. The entire full length construct was sequenced; comparison with the sequence of the parent clones used to create the construct did not reveal any differences.</p> <p>The cDNA was placed into an RNA transcription vector and plus strand RNA was made <u>in vitro</u> from the cDNA. Transfection of marmosets with the RNA failed to generate HAV. Preparation of plus strand HAV RNA, synthesized <u>in vitro</u> from minus strand HAV RNA and poliovirus RNA polymerase, is in progress.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00473-01 LID
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Replication of Hepatitis A Virus <u>In Vivo</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: Jeffrey I. Cohen, M.D. Others: Ruth A. Karron, M.D. Stephen M. Feinstone, M.D. Robert H. Purcell, M.D.	Medical Staff Fellow NSRA Medical Officer Head, HV Section	LID, NIAID LID, NIAID LID, NIAID LID, NIAID
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Hepatitis Viruses Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 0.5	PROFESSIONAL: 0.4	OTHER: 0.1
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>We have begun a study to look for extra-hepatic sites of HAV replication during experimental infection. Marmosets and chimpanzees are inoculated orally with wild-type or cell culture-adapted (AGMK Pass 30) HM-175 HAV. At serial time points animals are anesthetized and specimens of blood, saliva, and stool are obtained. Biopsies are taken from buccal mucosa, tonsils, and liver. These specimens are being analyzed for the presence of HAV by cell culture, cDNA hybridization, and immunofluorescence.</p> <p>In addition, primary cell cultures have been established from biopsies of gingiva and skin from the chimpanzees. These cell cultures will be infected with HAV <u>in vitro</u> for use as genetically restricted targets. Peripheral blood lymphocytes are being obtained by leukopheresis from the animals at various time points during infection. Incubation of these lymphocytes with the autologous HAV-infected target cells may indicate whether cellular immunity plays a role in the clearance of HAV-infected cells. Liver biopsies from the animals will be stained with antisera to lymphocyte markers (OK T4, OK T8, etc.) to determine which cell populations are associated with clearance of HAV infected hepatocytes.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00323-05 LID
PERIOD COVERED <u>October 1, 1985 to September 30, 1986</u>		
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.) <u>Structure of Parainfluenza Type 3 Virus Genome</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Peter L. Collins, Ph.D.	Senior Staff Fellow LID, NIAID
Others:	Melanie K. Spriggs, Ph.D.	Staff Fellow LID, NIAID
	Kathleen Coelingh, Ph.D.	Senior Staff Fellow LID, NIAID
	Robert A. Olmsted, Ph.D.	Staff Fellow LID, NIAID
	Alicia Buckler-White	Staff Fellow LID, NIAID
COOPERATING UNITS (if any)		
LAB/BRANCH <u>Laboratory of Infectious Diseases</u>		
SECTION <u>Respiratory Viruses Section</u>		
INSTITUTE AND LOCATION <u>NIAID, NIH, Bethesda, Maryland 20892</u>		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.7	1.2	0.5
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> We have constructed and identified cDNA clones of human parainfluenza type 3 virus (PIV3) mRNAs encoding the hemagglutinin-neuraminidase (HN) glycoprotein, fusion (F) glycoprotein, major nucleocapsid protein (NP), nucleocapsid phosphoprotein (P), and matrix (M) protein. The P mRNA contains a second, overlapping open reading frame that encodes the nonstructural (C) protein. Complete nucleotide sequences have been determined for the HN, F, NP, P+C and M mRNAs. Synthetic oligonucleotides were used to direct dideoxynucleotide sequencing of gene junctions in PIV3 genomic RNA (vRNA). From the sequencing of vRNA, a seventh viral gene was detected and was identified as the L gene by RNA blot hybridization. The order of the six PIV3 genes on vRNA is 3'-NP-P+C-M-F-HN-L. The five intergenic regions consist of the trinucleotide 3'-GAA. These studies show that PIV3 encodes six mRNAs (NP, P+C, M, F, HN and L) that encode seven proteins (NP, P, C, M, F, HN, and L). Preliminary studies on the expression of the F and HN genes by recombinant vaccinia viruses and SV40 viruses are described, together with the construction of new SV40 vectors and a new strategy for rapid mutagenesis and expression. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 A1 00324-05 L1D
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Laboratory Studies of Myxoviruses		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)		
PI:	Mark H. Snyder, M.D.	Medical Staff Fellow L1D, NIAID
Others:	Alicia Buckler-White	Staff Fellow L1D, NIAID
	Brian R. Murphy, M.D.	Head, RV Section L1D, NIAID
	Robert M. Chanock, M.D.	Chief L1D, NIAID
COOPERATING UNITS (if any) U. of Michigan (Dr. Maassab, Dr. DeBorde)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Respiratory Viruses Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS	PROFESSIONAL:	OTHER
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unindented type. Do not exceed the space provided.) <p> Studies with reassortant viruses which contained human influenza A virus surface antigens from the A/Washington/80 (H3N2) virus and one or more internal genes from the avian A/Pintail/Alberta/79 virus demonstrated: (1) a particular constellation of polymerase genes specifies a host range dependent restriction of replication in MDCK but not chick kidney tissue culture, (2) the ability to replicate at 42°C is a complex, multigene-associated phenotype, and (3) the NP gene is the major contributor to attenuation in monkeys. </p> <p> Comparison of the nucleotide and amino acid sequences of the NP genes of the avian A/Mallard/NY/78 with those of two other avian and three human influenza A viruses showed the existence of separate avian and human classes of NP genes with separate evolutionary patterns. The rate of evolution of human influenza A virus NP genes in the first several years immediately following the emergence of a new subtype in 1968 was increased compared with other years. </p> <p> Studies with single gene reassortant viruses derived from the human influenza A/Ann Arbor/6/60 <u>ca</u> virus confirmed the role of polymerase PA in specifying the <u>ca</u> phenotype and that of the polymerase PB2 in specifying the <u>ts</u> phenotype. The PA polymerase and M genes appear to play a role in attenuation. The mechanism of attenuation by the M gene is independent of <u>ts</u> or <u>ca</u> phenotypic markers. </p> <p> Reassortant viruses which contained a wild type influenza A/Alaska/77 virus NS gene which had sustained a 36 base deletion mutation in the region coding for the NS1 protein were studied. In association with "internal genes" from the <u>ca</u> donor virus, the mutant gene specified: (a) a host range restriction of replication in MDCK but not in chick kidney tissue culture, and (b) a <u>ts</u> phenotype. A reassortant containing the mutant NS in association with wild type genes exhibited only the <u>ts</u> phenotype. Loss of the <u>ts</u> phenotype occurred after replication of this reassortant in hamsters and chimpanzees. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00325-05 LID
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Study of Respiratory Viruses in Primates		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: Brian R. Murphy, M.D. Others: Mark H. Snyder, M.D. Kathleen L. Coelingh, Ph.D. Robert M. Chanock, M.D.	Head, RV Section Medical Staff Fellow Senior Staff Fellow Chief	LID, NIAID LID, NIAID LID, NIAID LID, NIAID
COOPERATING UNITS (if any) Meloy Laboratories (Dr. Jere Philips), Rockville, MD; NINCDS, NIH (Dr. William T. London); WRAIR, Division of Aerobiology (Dr. Edward Stevenson) Frederick, MD		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Respiratory Viruses Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 0.8	PROFESSIONAL: 0.4	OTHER: 0.4
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The nucleoprotein (NP) gene, but not the M gene, of the A/Pintail/119/79 avian influenza A virus specifies attenuation in monkeys. The PB2 gene also contributes to attenuation, but the restriction of replication specified by this gene can be modified by the presence of other avian or human influenza A virus genes. A reassortant lacking the attenuating NP and PB2 avian influenza virus genes, but containing the other four avian influenza A virus genes was also attenuated in monkeys. Thus, genes other than NP and PB2 can also contribute to the attenuation phenotype.</p> <p>The infectious dose₅₀ of an avian-human influenza A virus reassortant virus for squirrel monkeys was comparable when delivered by either aerosol or intranasal route. This suggests that the aerosol route of administration of viruses that are restricted in their replication in the lungs has little advantage over the intranasal route.</p> <p>Bovine parainfluenza virus type 3 is attenuated for chimpanzees and induces a high level of resistance to infection with human parainfluenza type 3 virus in squirrel monkeys. These observations suggest that the bovine parainfluenza type 3 virus may prove to be useful in immunization against human parainfluenza type 3 virus disease during infancy and early childhood.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00326-05 LID
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Study of Respiratory Viruses in Volunteers		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Mark H. Snyder, M.D. Medical Staff Fellow	LID, NIAID
Others:	Brian R. Murphy, M.D. Head, RV Section Robert M. Chanock, M.D. Chief	LID, NIAID LID, NIAID
COOPERATING UNITS (if any) Flow Labs, Rockv. MD (Potash); U Md. S. Med. Balt. MD (Clements); Johns Hop. U. Balt. MD (Clements); U Roch. S. Med. Roch. NY (Betts, Dolin); Vanderbilt U S. Med. Nashv. TN (Wright); Marshall U. S. Med. Huntington, WVA (Belshe); FDA, Beth. MD. (Burlington); NCI/NIH, Beth. MD (Wagner, Nelson)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Respiratory Viruses Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.9	0.6	2.3
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)		
<p>The six "internal" genes of an additional avian influenza donor virus, A/Mallard/Alberta/88/76, attenuated the wild-type human influenza A/Korea/1/82 virus for humans. In terms of attenuation, safety, restriction of virus replication and immunogenicity, this avian-human influenza A reassortant virus was similar to the previously studied human-avian influenza A reassortant viruses produced using the avian influenza A/Mallard/NY/6750/78 donor virus.</p> <p>Studies with reassortant viruses which contain a single RNA segment derived from the influenza A/Ann Arbor/6/60 <u>ca</u> donor virus and all other RNA segments from the influenza A/Korea/82 virus confirm our hypothesis that the polymerase PA of the <u>ca</u> donor virus plays a major role in the transfer of the <u>ca</u> and attenuation phenotypes. These studies indicate that the attenuation specified by the polymerase PA is due to a mechanism other than temperature sensitivity.</p> <p>Nasal wash HA specific IgG antibody detected in volunteers who received live or inactivated influenza A vaccines was derived as a transudate from serum. The minimum serum HA specific IgG ELISA titer required to produce detectable nasal wash antibody, 1:350, is similar to the level of transplacentally-derived serum neutralizing antibody that is associated with resistance to another virus, respiratory syncytial virus.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00327-05 LID
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Characterization of Parainfluenza and RS Virus Surface Glycoproteins		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Kathleen L. vanWyke Coelingh, Ph.D. Senior Staff Fellow LID, NIAID Others: Judith A. Beeler, M.D. IPA LID, NIAID Brian R. Murphy, M.D. Head, RV Section LID, NIAID Peter L. Collins, Ph.D. Senior Staff Fellow LID, NIAID		
COOPERATING UNITS (if any) Battelle Memorial Institute, Columbus, OH (Drs. J. Rice and P. Kimball); New York Univ., NY, NY (E. Jorgenson); Karolinska Institute, Stockholm, Sweden (Dr. E. Norrby); CDC, Atlanta, GA (Dr. L. Anderson); Univ. Rochester, Rochester, NY (Dr. E. Walsh)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Respiratory Viruses Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 2.8	PROFESSIONAL: 1.8	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>We have expanded the operational epitope map and functional map of the type 3 parainfluenza virus (PIV3) hemagglutinin-neuraminidase (HN) protein. Monoclonal antibodies (mAbs) to the HN protein define 14 operationally unique epitopes which are organized into 5 topographically non-overlapping antigenic sites (A,B,D,E, and F) and one bridging site (C). MAbs to sites A,B, and C inhibit hemagglutination and infectivity, and several site A mAbs also inhibit sialidase activity. MAbs to sites D, E, and F do not inhibit any known biological activity and react with all but 1 of 37 clinical PIV3 isolates examined, which is in contrast to mAbs to more variable epitopes in sites A,B, and C. Sequence analysis of HN genes of 16 mAb-resistant antigenic variants indicate the HN epitopes are located in hydrophilic stretches of amino acids. Computer analysis predicts these amino acids to form hydrophilic loops which connect B-sheet structures. Antigenic variants selected with mAbs which cross-react with the bovine PIV3 have amino acid substitutions in residues which are conserved in the primary structures of the parental human and bovine strains.</p> <p>MAbs to the fusion and G surface glycoproteins of respiratory syncytial virus have also been produced and are being characterized prior to selection of antigenic variants, epitope mapping, and sequence analysis of variants.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00344-05 LID
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Immunologic Factors in Respiratory Syncytial Virus (RSV) Disease		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: Gregory A. Prince, D.D.S., Ph.D.	Expert	LID, NIAID
Others: Robert M. Chanock, M.D. Brian R. Murphy, M.D.	Chief, LID Head, RVS, LID	LID, NIAID LID, NIAID
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Respiratory Viruses Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 1.4	PROFESSIONAL: 0.4	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The potentiation of RSV lower respiratory tract disease by prior inoculation of formalin-inactivated RSV vaccine which occurred during clinical trials of the vaccine 20 years ago was reproduced in the laboratory for the first time. Potentiation of pulmonary pathology was observed when cotton rats (<i>Sigmodon hispidus</i>) previously immunized with formalin-inactivated RSV vaccine underwent a RSV infection of their respiratory tract. Within 24 hours after infection with RSV, immunized cotton rats developed exaggerated pulmonary lesions that reached a maximum by day 4. Histologically, the lesions resembled an experimental pulmonary Arthus reaction. An action of formalin on RSV appears to be responsible for this effect, because live virus or virus heated in the absence of formalin did not induce enhanced immunopathology. Epitopes on the fusion (F) and/or attachment (G) RSV surface glycoproteins involved in inducing neutralizing antibodies were modified by formalin resulting in a reduction or ablation of a neutralizing antibody response. Nonetheless cotton rats inoculated parenterally with formalin-inactivated virus developed a high level of F and G antibodies measurable by an enzyme-linked immunosorbent assay indicating that most of the epitopes not involved in neutralization were not altered by formalin. At this time, the effect of formalin on RSV cannot be localized to either the F or G glycoprotein of RSV. Staining of lung sections from vaccinated, infected animals showed abundant deposits of IgG and C₃, indicating a major role for a type III (Arthus) immunopathologic reaction. </p> <p> Subsequent studies in two other species of cotton rats (<i>S. fulviventer</i> and <i>S. arizonae</i>), one inbred strain of mice (DBA/2N) and owl monkeys have all shown a similar pattern of disease enhancement in animals pretreated with formalin-killed virus. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00345-05 LID
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Immunity to RS Virus		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
Gregory Prince, D.D.S., Ph.D.	Expert	LID, NIAID
Robert M. Chanock, M.D. Brian R. Murphy, M.D.	Chief Head, RVS	LID, NIAID LID, NIAID
COOPERATING UNITS (if any) USUHS, Bethesda, MD (Dr. Hemming): Childrens Hospital National Medical Center, Wash., D.C. (Dr. Rodriguez).		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Respiratory Viruses Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 1.4	PROFESSIONAL: 0.4	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)		
<p>Human convalescent antiserum to respiratory syncytial virus (RSV) administered intraperitoneally to cotton rats prior to RSV challenge provided near-complete protection from pulmonary infection. Antiserum given subsequent to viral challenge at the peak of virus replication, reduced pulmonary viral titer 10^4 or greater within 24 hours. Sandoglobulin, a preparation of purified human IgG with high titer of RSV neutralizing antibodies, produced the same effects as convalescent antiserum. Sandoglobulin was absorbed rapidly and produced a significant therapeutic reduction in virus titer within 3 hours. The degree of reduction of virus in pulmonary and nasal tissues was directly proportional to the titer of neutralizing antibodies attained in the serum of the recipient cotton rat and the extent of reduction was always greater in the lungs than the nose.</p> <p>Studies in the owl monkey showed that the intravenous administration of RSV antibodies at the time of maximum viral shedding reduced pulmonary viral titer an average of $10^{1.5}$ within 48 hours, and cleared detectable virus from 75% of the animals. In spite of relatively high doses of antibody (3 gm/Kg) the treatment was well tolerated and did not exacerbate pulmonary disease. On the basis of these two studies, clinical trials of antibody therapy in infants hospitalized with RSV pneumonia are currently in progress at Children's Hospital National Medical Center.</p> <p>Therapy of RSV lower respiratory infection can also be achieved by administration of RSV antibodies directly into the lungs. The amount of antibodies required for a therapeutic effect is one three hundredth that needed when RSV antiserum is administered parenterally.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00368-04 LID																					
PERIOD COVERED October 1, 1985 to September 30, 1986																							
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Structural Analysis of Respiratory Syncytial Virus Genome																							
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: Peter L. Collins, Ph.D.</td> <td style="width: 33%;">Senior Staff Fellow</td> <td style="width: 33%;">LID, NIAID</td> </tr> <tr> <td colspan="3"> </td> </tr> <tr> <td>Others: Robert A. Olmsted, Ph.D.</td> <td>Staff Fellow</td> <td>LID, NIAID</td> </tr> <tr> <td>Melanie K. Spriggs, Ph.D.</td> <td>Staff Fellow</td> <td>LID, NIAID</td> </tr> <tr> <td>Alicia Buckler-White</td> <td>Staff Fellow</td> <td>LID, NIAID</td> </tr> <tr> <td>Kathleen Coelingh, Ph.D.</td> <td>Senior Staff Fellow</td> <td>LID, NIAID</td> </tr> <tr> <td>Philip R. Johnson, M.D.</td> <td>IPA</td> <td>LID, NIAID</td> </tr> </table>			PI: Peter L. Collins, Ph.D.	Senior Staff Fellow	LID, NIAID				Others: Robert A. Olmsted, Ph.D.	Staff Fellow	LID, NIAID	Melanie K. Spriggs, Ph.D.	Staff Fellow	LID, NIAID	Alicia Buckler-White	Staff Fellow	LID, NIAID	Kathleen Coelingh, Ph.D.	Senior Staff Fellow	LID, NIAID	Philip R. Johnson, M.D.	IPA	LID, NIAID
PI: Peter L. Collins, Ph.D.	Senior Staff Fellow	LID, NIAID																					
Others: Robert A. Olmsted, Ph.D.	Staff Fellow	LID, NIAID																					
Melanie K. Spriggs, Ph.D.	Staff Fellow	LID, NIAID																					
Alicia Buckler-White	Staff Fellow	LID, NIAID																					
Kathleen Coelingh, Ph.D.	Senior Staff Fellow	LID, NIAID																					
Philip R. Johnson, M.D.	IPA	LID, NIAID																					
COOPERATING UNITS (if any)																							
LAB/BRANCH Laboratory of Infectious Diseases																							
SECTION Respiratory Viruses Section																							
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892																							
TOTAL MAN-YEARS: 2.4	PROFESSIONAL: 1.8	OTHER: 0.6																					
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews																							
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> Previously, complete cDNAs and complete nucleotide sequences were obtained for nine of the ten known viral mRNAs of human respiratory syncytial virus (RSV) strain A2. Here, synthetic oligodeoxynucleotides were used to direct dideoxynucleotide sequencing of intergenic and flanking regions in the viral genome (vRNA). Comparison of the intergenic and flanking sequences with the complete mRNA sequences established unambiguously the 3' to 5' order of the nine genes on vRNA, 3'-NS1-NS2-N-P-M-SH-G-F-22K-L. Each gene was immediately followed (in genome-sense) by an oligo U tract of 4-7 residues that might direct synthesis of poly A tails of the mRNAs by a reiterative copying mechanism. The intergenic regions varied in length from 1 to 52 nucleotides and displayed no obvious sequence conservation except that in all cases the last nucleotide (vRNA-sense) was an A residue. The sequence of the first 1010 nucleotides of the L gene was determined by dideoxynucleotide sequencing of vRNA. Mapping and sequencing of the 5' end of the L mRNA established that, unexpectedly, the L gene overlaps with its upstream neighbor, the 22K gene. Specifically, the first 68 nucleotides of the L mRNA are identical to the last 68 nucleotides of the 22K mRNA, and are encoded by the same vRNA sequence. This genetic arrangement is without precedent among negative strand viruses. Further analysis of the transcription products representing this vRNA region is described. Finally, cDNA cloning and sequence analysis have been initiated for two additional RSV strains, the Long and 18537 strains. These have been shown by others to be distinct from the previously characterized A2 strain on the basis of reactivity with a panel of monoclonal antibodies to proteins of the envelope and nucleocapsid. Information on the structural and antigenic heterogeneity of RSV will be important in designing appropriate vaccine strategies. </p>																							

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00372-04 LID																					
PERIOD COVERED October 1, 1985 to September 30, 1986																							
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Respiratory Syncytial Virus Glycoproteins																							
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: Peter L. Collins, Ph.D.</td> <td style="width: 33%;">Senior Staff Fellow</td> <td style="width: 33%;">LID, NIAID</td> </tr> <tr> <td>Others: Robert A. Olmsted, Ph.D.</td> <td>Staff Fellow</td> <td>LID, NIAID</td> </tr> <tr> <td>Philip R. Johnson, M.D.</td> <td>IPA</td> <td>LID, NIAID</td> </tr> <tr> <td>Gregory A. Prince, Ph.D.</td> <td>Expert</td> <td>LID, NIAID</td> </tr> <tr> <td>Lewis J. Markoff, M.D.</td> <td>Senior Investigator</td> <td>LID, NIAID</td> </tr> <tr> <td>Brian R. Murphy, M.D.</td> <td>Head, RV Section</td> <td>LID, NIAID</td> </tr> <tr> <td>Robert M. Chanock, M.D.</td> <td>Chief</td> <td>LID, NIAID</td> </tr> </table>			PI: Peter L. Collins, Ph.D.	Senior Staff Fellow	LID, NIAID	Others: Robert A. Olmsted, Ph.D.	Staff Fellow	LID, NIAID	Philip R. Johnson, M.D.	IPA	LID, NIAID	Gregory A. Prince, Ph.D.	Expert	LID, NIAID	Lewis J. Markoff, M.D.	Senior Investigator	LID, NIAID	Brian R. Murphy, M.D.	Head, RV Section	LID, NIAID	Robert M. Chanock, M.D.	Chief	LID, NIAID
PI: Peter L. Collins, Ph.D.	Senior Staff Fellow	LID, NIAID																					
Others: Robert A. Olmsted, Ph.D.	Staff Fellow	LID, NIAID																					
Philip R. Johnson, M.D.	IPA	LID, NIAID																					
Gregory A. Prince, Ph.D.	Expert	LID, NIAID																					
Lewis J. Markoff, M.D.	Senior Investigator	LID, NIAID																					
Brian R. Murphy, M.D.	Head, RV Section	LID, NIAID																					
Robert M. Chanock, M.D.	Chief	LID, NIAID																					
COOPERATING UNITS (if any) <div style="text-align: center;">LVD, NIAID (Dr. B. Moss, Dr. N. Elango)</div>																							
LAB/BRANCH Laboratory of Infectious Diseases																							
SECTION Respiratory Viruses Section																							
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892																							
TOTAL MAN-YEARS	1.6	PROFESSIONAL: 1.4																					
		OTHER: 0.2																					
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews																							
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Previously, complete sequences for the mRNAs encoding the RSV G and F glycoproteins were determined from cDNA clones (annual reports, 1984, 1985, and Satake et al., 1985). Here we constructed vaccinia virus recombinants (vaccinia-F and vaccinia-G) that express the individual RSV glycoproteins. Vaccinia-F and vaccinia-G were administered to cotton rats to evaluate the relative contributions of the individual glycoproteins to host immunity. Both recombinants induced high levels of RSV serum antibodies, measured both by ELISA and by RSV neutralization plaque reduction. Upon subsequent intranasal (IN) challenge with RSV, vaccinia-G provided substantial protection in the lungs and no protection in the nose, and vaccinia-F provided complete protection in the lungs and partial protection in the nose. Importantly, the serum neutralizing antibody titers induced by vaccinia-F were slightly higher than that induced by RSV infection of the respiratory tract, and 6-fold higher than obtained with vaccinia-G. Thus, the F glycoprotein appears to be more effective in stimulating host immunity. These results show that the vaccinia virus recombinants should be evaluated further as potential methods of immunoprophylaxis. Also, the cotton rat was found to be a sensitive assay for testing the safety of vaccinia virus recombinants. The F and G cDNAs have also been expressed in tissue culture using recombinant SV40 viruses. In conjunction with site-directed mutagenesis, these studies will define amino acid sequences that are important in protein function.</p>																							

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00457-02 LID																					
PERIOD COVERED October 1, 1985 to September 30, 1986																							
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Human Immune Response to Respiratory Syncytial Virus Infection or Vaccination																							
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: Brian R. Murphy, M.D.</td> <td style="width: 33%;">Head, RV Section.</td> <td style="width: 33%;">LID, NIAID</td> </tr> <tr> <td>Others: Gregory Prince, D.D.S.</td> <td>Expert</td> <td>LID, NIAID</td> </tr> <tr> <td>Judy A. Beeler, M.D.</td> <td>IPA</td> <td>LID, NIAID</td> </tr> <tr> <td>Kathleen Coelingh, Ph.D.</td> <td>Senior Staff Fel.</td> <td>LID, NIAID</td> </tr> <tr> <td>Robert M. Chanock, M.D.</td> <td>Chief</td> <td>LID, NIAID</td> </tr> <tr> <td>Mark H. Snyder, M.D.</td> <td>Medical Staff Fel.</td> <td>LID, NIAID</td> </tr> <tr> <td>David Alling</td> <td></td> <td>OSD, NIAID</td> </tr> </table>			PI: Brian R. Murphy, M.D.	Head, RV Section.	LID, NIAID	Others: Gregory Prince, D.D.S.	Expert	LID, NIAID	Judy A. Beeler, M.D.	IPA	LID, NIAID	Kathleen Coelingh, Ph.D.	Senior Staff Fel.	LID, NIAID	Robert M. Chanock, M.D.	Chief	LID, NIAID	Mark H. Snyder, M.D.	Medical Staff Fel.	LID, NIAID	David Alling		OSD, NIAID
PI: Brian R. Murphy, M.D.	Head, RV Section.	LID, NIAID																					
Others: Gregory Prince, D.D.S.	Expert	LID, NIAID																					
Judy A. Beeler, M.D.	IPA	LID, NIAID																					
Kathleen Coelingh, Ph.D.	Senior Staff Fel.	LID, NIAID																					
Robert M. Chanock, M.D.	Chief	LID, NIAID																					
Mark H. Snyder, M.D.	Medical Staff Fel.	LID, NIAID																					
David Alling		OSD, NIAID																					
COOPERATING UNITS (if any) U. Roch. Sch. Med. Roch., N.Y. (Walsh); Childrens Hosp. Nat. Med. Cen. Wash., DC. (Kim, Parrot); NCI/NIH, Beth. MD. (Nelson, Wagner); Vanderbilt U. Nashville, TN (Wright); CDC Atlanta, GA (Reimer); USUHS Beth., MD. (Hemming); BOB, FDA, Beth. MD (Hendry); Northwestern U., Chic., Ill. (Lamb, Patterson)																							
LAB/BRANCH Laboratory of Infectious Diseases																							
SECTION Respiratory Viruses Diseases																							
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892																							
TOTAL MAN-YEARS: 0.9	PROFESSIONAL: 0.6	OTHER: 0.3																					
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews																							
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Young infants (<8 months of age) have decreased ELISA F and G and neutralizing antibody responses to respiratory syncytial virus (RSV) infection. Age primarily affects the response to the F glycoprotein and pre-existing antibody affects the response to the G glycoprotein. Infants and children less than two years of age primarily see the heavily glycosylated G glycoprotein as a protein antigen, i.e., they mount an ELISA IgG1 and IgG3 subclass response to the G glycoprotein. Adults, however, generate an IgG2 response equivalent to their IgG1 response. Thus, the G protein can be seen by the immune system as a protein and/or carbohydrate antigen.</p> <p>Infants and children immunized with formalin-inactivated RSV vaccine developed titers of ELISA antibodies equivalent to those of individuals infected with RSV, but the neutralizing antibody titers of the vaccinees were low. Thus, treatment of RSV with formalin appears to have altered the epitopes of the F and/or G glycoproteins that stimulate neutralizing antibodies with the result that the immune response to vaccine consisted largely of "nonfunctional" (i.e., non-neutralizing) antibodies.</p> <p>In tests employing post-infection sera of young infants the Long and 18537 strains of RSV were shown to differ by approximately 3 fold in cross neutralization assays. The G glycoproteins were about 8-fold different by ELISA and the F proteins about 2-fold different.</p> <p>Both the HN and F glycoproteins of a parainfluenza type 2 virus (SV-5) (in a Vaccinia-SV-5 recombinant) induced SV-5 neutralizing antibodies and resistance to SV-5 virus challenge.</p>																							

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00474-01 LID
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Molecular Mechanisms Underlying Adenovirus Virulence		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: Harold S. Ginsberg, M.D.	IPA	LID, NIAID
Others: Robert M. Chanock, M.D. Gregory Prince, D.D.S., Ph.D. Jorge Flores, M.D. Kim Green, Ph.D.	Chief Expert Visiting Scientist Staff Fellow	LID, NIAID LID, NIAID LID, NIAID LID, NIAID
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Respiratory Virus Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 1.2	PROFESSIONAL: 1.2	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> Research has been initiated to determine the adenovirus gene products required for cell damage and to establish the molecular mechanisms effecting the pathogenesis of adenovirus diseases. The cotton rat is an ideal animal model for these studies because the pathology of the pneumonia and upper respiratory disease produced by type 5 adenovirus in this animal is similar to that observed in humans. Basic experiments have been carried out to establish the parameters of the infection: kinetics of viral replication and clearance of virus from the lung; the temporal relationship of viral replication to the development of pneumonia; the cellular sites of viral replication in the lung; the immune response to infection; and the establishment of viral latency. Two species of cotton rats, <u>Sigmadon hispidus</u> and <u>Sigmadon fulviventir</u>, were compared in these studies; the <u>S. hispidus</u> proved to be the better species for investigating pathogenesis of pneumonia, but the <u>S. fulviventir</u>, appears to have distinct advantage for studies of the latent state. Experiments have been initiated using a variety of deletion, insertion, and conditionally lethal mutants in an effort to determine the gene product or gene products responsible for cell damage and subsequent development of pneumonia. </p> <p> An adenovirus expression vector, using the vaccine strain of type 4 adenovirus, is being constructed to develop an alternate viral vaccine containing the rotavirus gene encoding the major antigen that induces neutralizing Abs. This vaccine would have a number of advantages for prevention of rotavirus infections throughout the world. </p>		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00329-04 LID
TERMINATED

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Expression of the Influenza A Virus Neuraminidase Glycoprotein from Cloned DNA

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Lewis Markoff, M.D. Medical Officer LID, NIAID

Others: Ching-Juh Lai, Ph.D. Head, MVB Section LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Molecular Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

0.1

PROFESSIONAL:

0.1

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A full-length ds DNA copy of the virion RNA segment coding for an influenza A neuraminidase (NA) glycoprotein was previously cloned into the late (deleted) region of an SV40 shuttle vector. The influenza-specific product of a lytic infection with this vector was shown to be glycosylated and inserted in the outer cell membrane. Additional studies established that weak enzymatic activity of the vector-coded NA was detectable in lysates of infected cells. Three deletion mutant NA DNAs that lacked sequences coding for 7 (dlK), 21 (dlI) or all 23 amino acids (dlZ) of the N-terminal hydrophobic region in the wild-type NA were studied in similar fashion, and a comparison of the phenotypes of these mutants suggested that this region functions not only in membrane anchorage but also as a signal sequence, permitting entry of the nascent NA polypeptide into membrane organelles for glycosylation. Experiments are now in progress to induce point mutations in DNA coding for the hydrophobic N-terminus of the NA protein to determine whether alterations in this region may result in: (1) a membrane anchorage defect which would result in secretion of the mutant polypeptide, (2) altered processing as indicated by a change of glycosylation pattern, or (3) altered transport.

MERGED WITH Z01 AI 40803
TERMINATED 1986

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00332-04 LID
PERIOD COVERED October 1, 1985 to September 30, 1986		INACTIVE
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Engineering the Genome of Influenza Virus		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: Ching-Juh Lai, Ph.D.	Head, MVB Section	LID, NIAID
Others: Lewis J. Markoff, M.D.	Medical Officer	LID, NIAID
Kevin Ryan, Ph.D.	Staff Fellow	LID, NIAID
Erich Mackow, Ph.D.	Staff Fellow	LID, NIAID
Robert M. Chanock, M.D.	Chief	LID, NIAID
COOPERATING UNITS (if any) <div style="text-align: center; margin-top: 10px;"> The Wistar Inst., Philadelphia, Pa. (Jonathan Yewdell) </div>		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Molecular Viral Biology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 0.1	PROFESSIONAL: 0.1	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <div style="margin-top: 10px;"> <p>Our goal has been to use recombinant DNA techniques to construct influenza virus mutants with deletions in strategic regions of the genome. Viable deletion mutants would be especially valuable for use in immunoprophylaxis since these mutants would be unlikely to revert and therefore should be stable as regards phenotype. With this goal in mind, we produced full-length cloned DNA sequences of gene segments of an H3N2 influenza A virus. Thus far we have cloned and characterized 6 full-length genes (hemagglutinin (HA), neuraminidase (NA), nucleoprotein (NP), matrix protein (M), non-structural proteins (NS), and polymerase protein PB2): the remaining two genes (PB1 and PA) have also been cloned but not in complete form. These full-length DNA clones should produce corresponding RNA transcripts that contain the control sequences needed for transcription and replication of viral genes. The validity of this prediction was established for transcription and expression of viral proteins. Functional influenza viral protein (HA, NA, or NP) was produced when simian cells were transfected with a SV40 recombinant vector containing cloned influenza DNA. The influenza cDNA was inserted into the late region of SV40 in an orientation which resulted in transcription of (+) strand influenza RNA. Attempts to rescue cloned influenza DNA by coinfection of transfected cells with influenza A virus were unsuccessful.</p> </div> <div style="text-align: center; margin-top: 20px;"> <u>INACTIVE 1986</u> </div> <div style="text-align: center; margin-top: 20px;"> 8-60 </div>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00365-03 LID INACTIVE												
PERIOD COVERED October 1, 1985 to September 30, 1986														
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Functional Analysis of Signal Sequences of Influenza Virus Hemagglutinin														
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: Ching-Juh Lai, Ph.D.</td> <td style="width: 33%;">Head, MVB Section</td> <td style="width: 33%;">LID, NIAID</td> </tr> <tr> <td>Others: Lewis Markoff, M.D.</td> <td>Medical Officer</td> <td>LID, NIAID</td> </tr> <tr> <td>Alicia Buckler-White, Ph.D.</td> <td>Staff Fellow</td> <td>LID, NIAID</td> </tr> <tr> <td>Brian R. Murphy, M.D.</td> <td>Head, RV Section</td> <td>LID, NIAID</td> </tr> </table>			PI: Ching-Juh Lai, Ph.D.	Head, MVB Section	LID, NIAID	Others: Lewis Markoff, M.D.	Medical Officer	LID, NIAID	Alicia Buckler-White, Ph.D.	Staff Fellow	LID, NIAID	Brian R. Murphy, M.D.	Head, RV Section	LID, NIAID
PI: Ching-Juh Lai, Ph.D.	Head, MVB Section	LID, NIAID												
Others: Lewis Markoff, M.D.	Medical Officer	LID, NIAID												
Alicia Buckler-White, Ph.D.	Staff Fellow	LID, NIAID												
Brian R. Murphy, M.D.	Head, RV Section	LID, NIAID												
COOPERATING UNITS (if any) Laboratory of Immunogenics, NIAID, NIH, Bethesda, MD. (Dr. John Coligan)														
LAB/BRANCH Laboratory of Infectious Diseases														
SECTION Molecular Viral Biology Section														
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892														
TOTAL MAN-YEARS: 0.1	PROFESSIONAL: 0.1	OTHER: 0.0												
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews														
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The amino acid requirements of a functional influenza virus signal peptide were investigated using the influenza hemagglutinin (HA) cDNA-SV40 expression system in African green monkey kidney (AGMK) cells. Local site-specific mutagenesis was carried out to generate a series of recombinants of HA-SV40 containing point mutations in the region of the influenza virus hemagglutinin (HA) gene that codes for the signal peptide sequences. These mutant HA-SV40 recombinants were used to transfect AGMK cells in order to achieve expression of mutant hemagglutinins. Functional characterization of such HA products by cell surface immunofluorescence assay, hemadsorption and analysis of glycosylation showed that a majority of the mutations had no effect on functional properties of HA. However, one isolate (mutant 28) that sustained several mutations including an amino acid substitution at the signal cleavage site was defective with regard to cell surface expression. Amino acid sequence analysis of the NH₂-terminus of mutant HA showed that the intracellularly accumulated HA failed to undergo signal cleavage. Also, the defective mutant HA contained only endoglycosidase H sensitive carbohydrate components that are added in the endoplasmic reticulum. These findings suggest that HA containing an uncleaved hydrophobic signal sequence translocates across the microsomal membrane but fails to proceed to the Golgi apparatus where endoglycosidase H resistant carbohydrates are incorporated. Point mutagenesis using a defined oligonucleotide primer has been attempted with the intention of isolating a specific cleavage mutant that will allow us to confirm that the signal cleavage defect present in mutant 28 is indeed responsible for its defect in HA translocation and cell surface expression.</p>														
INACTIVE 1986 8-61														

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER 201 AI 00366-04 LID
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (90 characters or less. Title must fit on one line between the borders.) Molecular Biology of Dengue Viruses		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
Co-PI:	Banghti Zhao, Ph.D.	LID, NIAID
	Ching-Juh Lai, Ph.D.	LID, NIAID
Others:	Robert M. Chanock	Chief, LID
		LID, NIAID
COOPERATING UNITS (if any) Dept. Virus Diseases, Walter Reed Army Inst. of Research, Washington, D.C. (Dr. Donald Burke)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Molecular Viral Biology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205		
TOTAL MAN-YEARS:	1.4	PROFESSIONAL: 1.2
		OTHER: 0.2
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> We employed recombinant DNA techniques to investigate the molecular biology of dengue virus with the intent of developing immunoprophylactic measures against this virus that is epidemic in many geographic areas. DNA sequences (approximately 11,000 nucleotides) representing the full-length genome of the dengue virus type 4 were cloned. The sequence of the first 2,429 nucleotides at the 5'-terminus, which includes the coding region for the structural proteins, has been determined. The virion structural proteins are encoded in one long open reading frame specifying a polyprotein precursor which is apparently proteolytically cleaved by a mechanism resembling that proposed for expression of structural proteins of other flaviviruses such as yellow fever (YF) and West Nile (WN) viruses. The N-terminus for each of the dengue virus structural proteins was tentatively assigned by homology alignment to the corresponding sequence of YF or WN virus. Comparison of sequence homology of structural proteins suggests that dengue virus is more closely related to WN virus than to YF virus or Murray Valley encephalitis virus. Finally, analysis of the extreme 5'- and 3'-terminal nucleotides of the dengue virus genome revealed sequences that may be involved in transcription, replication and packaging of viral RNA. Attempts are currently underway to determine if cloned full-length dengue DNA is infectious after introduction into permissive cells. If successful, it will then be possible to construct defined viral mutants that may be useful for biologic studies. Such mutants may also prove to be useful in the development of a safe, effective live attenuated vaccine for prevention of dengue disease. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00369-03 LID TERMINATED 1986
PERIOD COVERED October 1, 1984 to September 30, 1985		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Persistent Expression of Influenza Virus Polymerase Proteins from Cloned DNA		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Erich Mackow, Ph.D.	Staff Fellow LID, NIAID
Others:	Ching-Juh Lai, Ph.D. Kevin Ryan, Ph.D. Lewis Markoff, M.D.	Head, MVB Section LID, NIAID Staff Fellow LID, NIAID Medical Officer LID, NIAID
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Molecular Viral Biology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)		
TERMINATED 1986		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00407-03 LID
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Complementation of Influenza Mutants by Cloned Viral Genes		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Kevin Ryan, Ph.D.	Staff Fellow LID, NIAID
Others:	Ching-Juh Lai, Ph.D. Erich Mackow, Ph.D. Robert Chanock, M.D.	Head, MVB Section Staff Fellow Chief LID, NIAID LID, NIAID LID, NIAID
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Molecular Viral Biology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	1.0	0.3
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> Efforts to achieve persistent expression of influenza A virus cloned DNA in cells permissive for virus infection were initiated because such cells should be useful for investigation of the molecular biology of influenza virus and for isolation of specific viral mutants through complementation by the expressed gene. In this manner, naturally occurring or laboratory engineered mutants containing viable deletion mutations could be isolated for evaluation of their level of attenuation. Initially, simian cells permissive for influenza A virus infection were stably transformed with a full length cloned influenza A nucleoprotein gene under the control of an inducible metallothionein promoter and linked to a dihydrofolate reductase gene to facilitate selection of transformed cells. The transformed cells which were selected synthesized an influenza A viral nucleoprotein which was indistinguishable from the nucleoprotein synthesized in virus-infected cells with respect to molecular weight and intracellular localization. It was estimated that transformed (CV1-NP) cells produced only 1% of the amount of nucleoprotein synthesized in simian cells infected with influenza A virus. Nonetheless, when these cells were infected with influenza virus mutants which synthesized temperature sensitive nucleoprotein, protein expressed by the cloned gene was able to complement the synthesis of plus-strand and minus-strand viral RNA for some mutants and only plus-strand synthesis for other mutants. This indicated that the nucleoprotein expressed in the transformed cells from cloned influenza A virus nucleoprotein cDNA exhibited functional activity. Furthermore, under appropriate conditions CV1-NP cells complemented the replication of <u>ts</u> mutant viruses. This complementation effect is currently being analysed to gain a better understanding of the biological functions of the viral nucleoprotein and to develop a strategy for isolation of viable deletion mutants. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00408-03 LID
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Attempts at Allele Replacement of NA Gene of Influenza A Virus		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Lewis J. Markoff, M.D. Medical Officer LID, NIAID		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Molecular Viral Biology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 1.1	PROFESSIONAL: 0.9	OTHER: 0.2
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The genome of many plus-strand RNA viruses is infectious. In contrast, the genome of negative-strand or of double-stranded RNA viruses is not infectious. We are attempting to render a single gene segment of a negative strand RNA virus, influenza A virus, infectious by rescuing RNA copies of the gene prepared <u>in vitro</u> from cloned full-length DNA, in cells infected with an influenza A virus. To facilitate such a "rescue", we chose a gene that bears a host range mutation that has a selective advantage. A full-length ds DNA copy of the influenza A WSN virus NA RNA was cloned. A complete cDNA copy of the gene was then expressed in an SV40 vector as a fully functional NA glycoprotein. Having established that the NA DNA was functional, we subcloned the DNA into a plasmid vector containing promoter sequences for phage RNA polymerases. (+) strand copies of NA DNA were generated <u>in vitro</u>. These transcripts necessarily contain short sequences at 5' and 3' ends copied from vector DNA. In the rescue experiment, primary AGMK cells were infected with a suitable recipient influenza A virus strain. After infection, cells were transfected with (+) strand RNA transcripts at high multiplicity. After 16 to 18 hours, the medium and the AGMK cell monolayer were harvested and inoculated onto a MDBK cell monolayer. Growth in MDBK cells would indicate that "rescue" of the WSN NA gene had occurred. Thus far, this approach has not succeeded. We are re-engineering the transcription vector to produce discrete NA copies of WSN NA DNA that lack terminal non-influenza sequences. We also plan to repeat the experiment in continuous monkey cell lines that persistently produce the influenza nucleoprotein, in one case, or the three influenza RNA polymerases, in another case. Possible such transformed cell lines may facilitate the rescue of transfected RNA. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00458-02 LID
PERIOD COVERED <u>October 1, 1985 to September 30, 1986</u>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>Genetic Variations Among Dengue Viruses</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Yi-ming Zhang, M.D.	Visiting Fellow LID, NIAID
Others:	Ching-Juh Lai, Ph.D.	Head, MVB Section LID, NIAID
COOPERATING UNITS (if any)		
LAB/BRANCH		
Laboratory of Infectious Diseases		
SECTION		
Molecular Viral Biology Section		
INSTITUTE AND LOCATION		
NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	1.2	0.2
CHECK APPROPRIATE BOX(ES)		
<input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither		
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)		
<p>The dengue virus subgroup of the flavivirus family is comprised of 4 distinct serotypes (type 1 to type 4). Intratypic variations have been detected within several serotypes by oligonucleotide fingerprint analysis and by virus neutralization. Dengue type 4 variants from the Caribbean recovered during 1981-1982 appear to be different from the dengue 4 prototype (H241) isolated in the Phillipines in 1956 as well as recent isolates from Southeast Asia. During the past two years, dengue type 4 was the most frequent cause of dengue hemorrhagic shock in Bangkok, in contrast to the earlier epidemic pattern in which dengue type 2 viruses were most often implicated. For these reasons it is important to define in molecular terms: (a) genetic stability and diversity of dengue 4 viruses isolated over a 30 year interval and (b) the possible involvement of specific virus strains, i.e., "virulent viruses", in severe dengue disease. Two dengue type 4 variants, the prototype strain H241 and strain 2123 isolated from a hemorrhagic shock patient, were chosen for cloning and DNA sequence analysis. Several specific oligonucleotide primers corresponding to the established dengue 4 sequence (Dominica strain) were tested for their ability to prime reverse-transcription. At least one oligonucleotide effectively primed each dengue genomic RNA and yielded cDNA 5,000 nucleotides in length. These cDNA products will be cloned by the procedure established earlier. Cloned DNA will then be analyzed to determine the sequence coding for the two protective antigens, the envelope glycoprotein and the NS1 nonstructural protein. Finally, sequences will be compared in an attempt to identify hypervariable regions in the E and NS1 glycoproteins.</p>		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00459-02 LID

PERIOD COVERED

October 1, 1985 to September 31, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Expression of Dengue Virus Proteins Using Baculovirus as a Vector.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Erich Mackow, Ph.D. Staff Fellow LID, NIAID

Others: Ching-Juh Lai, Ph.D. Head, MVB Section LID, NIAID

COOPERATING UNITS (if any)

Texas A & M University (Dr. M. Summers)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Molecular Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

0.8

PROFESSIONAL:

0.6

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have recently completed cloning and sequencing the entire genome of dengue type 4 virus. The RNA genome of dengue type 4 virus is 10,644 nucleotides long and contains a single long open reading frame (ORF) encoding a polyprotein chain of 3386 amino acids. The positive strand RNA of dengue 4 serves as a messenger RNA for the synthesis of a polyprotein chain which is post-translationally cleaved into the full complement of virus proteins. We have initiated the expression of single or multiple dengue type 4 virus proteins from our cDNA clones using baculovirus as a vector. A DNA fragment encoding: (a) the C, M, and E structural proteins, (b) the NS1 nonstructural protein or (c) the NS5 nonstructural protein has been inserted into a baculovirus intermediate vector. Subsequently baculovirus-dengue recombinants were identified and purified by plaque isolation. Currently we are screening these baculovirus recombinants for the expression of dengue proteins.

Expressed proteins will be used to immunize animals for preparation of antisera and these antisera will in turn be used for identification of dengue viral proteins produced during infection. Some of the expressed proteins may also prove useful as immunogens for use in the immunoprophylaxis of dengue virus infection and disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00460-01 LID TERMINATED
PERIOD COVERED <u>October 1, 1985 to September 30, 1986</u>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>Identification and Gene Mapping of Dengue Viral Antigens</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Yoshihiro Makino, Ph.D.	Visiting Associate LID, NIAID
Others:	Bangti Zhao, Ph.D. Ching-Juh Lai, Ph.D.	Visiting Associate LID, NIAID Head, MVB Section LID, NIAID
COOPERATING UNITS (if any) <p style="text-align: center;">Laboratory of Immunogenetics, NIAID (Dr. John Colligan).</p>		
LAB/BRANCH <u>Laboratory of Infectious Diseases</u>		
SECTION <u>Molecular Viral Biology Section</u>		
INSTITUTE AND LOCATION <u>NIAID, NIH, Bethesda, Maryland 20892</u>		
TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
0.2	0.2	0.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Current information indicates that the flavivirus virion contains three protein components, i.e. the glycosylated envelope protein (E), the non-glycosylated matrix protein (M) and the capsid protein (C). These proteins are proteolytic products of a long polyprotein precursor that is translated from a genomic-length RNA species. The genes coding for these viral structural proteins are clustered at the 5' terminus. We sought to provide evidence that the dengue virus genome also contains one open-reading frame and that the encoded polyprotein is processed to yield the individual viral proteins found in the virion and in infected cells. Using polyclonal antisera for immunoprecipitation, we have identified three dengue virion components of 51Kd, 14Kd, and 8Kd respectively. These dengue-specific proteins are being prepared for determination of their amino-terminal amino acid sequence. In the meantime, cloned DNA segments located at the extreme 5'-end of the genome are being sequenced. These studies should enable us to determine the map positions of the genes that code for structural proteins. Also, the complete amino acid sequence of these structural proteins can be deduced from the cDNA sequence of their genes.</p> <p style="text-align: center; margin-top: 20px;"> MERGED WITH Z01 AI 36604 <u>TERMINATED 1986</u> </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00475-01 LID

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Nucleotide Sequence of Genes for Dengue Virus Type 4 Nonstructural Proteins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: E. R. Mackow, Ph.D. Staff Fellow LID, NIAID

Others: B. Zhao, Ph.D. Visiting Associate LID, NIAID

Y. Makino, Ph.D. Visiting Associate LID, NIAID

C.-J. Lai, Ph.D. Head, MVB Section LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Molecular Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

0.8

PROFESSIONAL:

0.6

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The single strand viral RNA genome of dengue type 4 virus has been completely sequenced. The genome contains 10,644 nucleotides with a single long open reading frame that encodes a polyprotein of 3386 amino acids. The polyprotein is cleaved post-translationally into the full complement of viral proteins by a mechanism similar to that proposed for gene expression of other flaviviruses. Homology alignment of the dengue type 4 virus polyprotein with the polyproteins of two other flaviviruses, yellow fever and West Nile viruses, indicated that the 5' end of the dengue viral RNA encodes three structural proteins designated capsid (C), matrix (M) and envelope (E). The 3' terminal 7500 nucleotides encode seven nonstructural (NS) proteins designated NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5. NS1 is the major NS glycoprotein produced during viral infection, while NS3 and NS5 contain polymerase-like amino acid sequences. Except for NS1, the function of dengue NS proteins is not known. Dengue NS proteins are more highly related to West Nile (WN) or Murray Valley encephalitis (MVE) virus NS proteins than to yellow fever (YF) virus NS proteins. In the region of the polyprotein containing the nonstructural proteins there is considerable conservation of hydrophobicity and a consensus sequence prevails at many proteolytic cleavage sites implying a close functional relationship for corresponding NS proteins. In view of the recent finding that the YF NS1 induces resistance in mice to challenge with YF virus, it is likely that the analogous dengue NS1 protein may prove useful in immunoprophylaxis against dengue infection.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00476-01 LID
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Engineering the Genome of Dengue Virus		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Astrid Breuning, Ph.D. Visiting Fellow, MVB Sec.	LID, NIAID
Others:	Ching-Juh Lai, Ph.D. Head, MVB Sec.	LID, NIAID
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Molecular Viral Biology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	1.2	0.2
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> A cloned full-length DNA copy of the dengue type 4 virus genome was obtained and its complete sequence analyzed. Experiments were then undertaken to determine if cloned dengue DNA, or an RNA transcript derived from it, is infectious after introduction into permissive cells. First, we introduced SV40 DNA sequences which represent strong signals for replication and transcription into the plasmid vector in an attempt to increase the infectivity of cloned dengue DNA. Second, because dengue viral RNA is infectious we have initiated efforts to introduce dengue RNA transcripts from cloned DNA directly into cultured cells. In these efforts the versatile <u>in vitro</u> transcription system of SP6 and T7 phages is being utilized. The full-length dengue DNA will be inserted into such a plasmid vector (Gemini 3) at the unique Pst I site. The dengue DNA recombinant will then be linearized with Kpn I thus making it possible to obtain "run-off" transcripts which can be assayed for infectivity. If we are successful, site-specific mutagenesis will be performed to construct dengue virus mutants containing mutations in strategic regions essential for viral replication and other gene functions. Dengue virus mutants constructed in this manner may exhibit an altered phenotype such as temperature-sensitivity of virus replication or reduced virulence for lower primates and humans. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00477-01 LID

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Expression of Dengue Viral Genes in Vaccinia Recombinant Virus

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Bangti Zhao, Ph.D. Visiting Associate LID, NIAID
Ching-Juh Lai, Ph.D. Head, MVB Section LID, NIAID

Others: Robert M. Chanock, M.D. Chief LID, NIAID

COOPERATING UNITS (if any)

Laboratory of Viral Diseases, NIAID (B. Moss)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Molecular Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

1.4

PROFESSIONAL:

1.2

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The initial goal in this project was to express both protective antigens of dengue virus, i.e., the E glycoprotein and the NS1 nonstructural glycoprotein from cloned dengue cDNA using vaccinia virus as a vector. Because these and other dengue proteins are presumably proteolytically cleaved from their polyprotein precursor, the 5' region of the dengue genomic cDNA that codes for all the structural proteins, (C, (PreM) M, and E) as well as the first two downstream nonstructural proteins, NS1 and NS2a, was inserted into vaccinia virus. In this construct the dengue cDNA insert is under the control of the P7.5 early-late vaccinia promoter, the vector contains the *E.coli* β -galactosidase gene under the control of a separate (P11) vaccinia promoter and both chimeric genes are flanked by vaccinia thymidine kinase (TK) sequences. Initially, dengue viral antigens were detected by indirect immunofluorescence in CV-1 cells infected with the vaccinia-dengue recombinant. Specific identification was made using a dengue type 4 virus E glycoprotein monoclonal or a polyvalent dengue type 4 virus antiserum prepared in mice. Vaccinia-dengue recombinant infected cells were then labelled with ³⁵S-methionine and the cell lysate was processed for immunoprecipitation in order to determine which dengue-specific viral products were expressed. SDS-polyacrylamide electrophoresis indicated that the dengue E glycoprotein monoclonal antibody precipitated a protein of approximately 50-55 Kilo daltons (Kd) molecular weight which is the expected size of the E glycoprotein. Similarly, a dengue type 2 virus NS1 antiserum precipitated a 35-38 Kd protein; this size is consistent with the molecular weight of the NS1 protein. These observations suggest that both glycoproteins were synthesized and specifically processed in a manner similar to that of authentic dengue proteins in virus infected cells. Currently, cotton rats are being immunized with the vaccinia-dengue recombinant.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00333-05 LID
PERIOD COVERED October 1, 1985 through September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) A Longitudinal Study of Viral Gastroenteritis in Infants and Young Children		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Albert Z. Kapikian, M.D. Head, Epid. Sect. LID, NIAID		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Epidemiology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS:	<0.1	PROFESSIONAL: <0.1 OTHER: 0.0
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Rotaviruses have been studied extensively predominantly by cross-sectional approaches. Such studies have yielded essentially "numerator" data which indicated that rotaviruses are a major cause of diarrheal illness. There have been few longitudinal gastroenteritis studies yielding important epidemiologic information. Therefore we initiated an examination of anal swab and serum specimens obtained during a previous long-term longitudinal study (1955-1969) at Junior Village, a welfare institution for normal, homeless children. Anal swabs and blood specimens were obtained routinely. Surveillance was carried out by a trained medical staff. As reported previously, 139 rotavirus strains were detected with the characteristic seasonal distribution. It should be possible to establish the serotypic diversity of these strains. The subgrouping pattern of tested strains was of special interest in that both subgroup 1 and subgroup 2 viruses were detected. In addition, as noted previously, sequential sera from 384 children in residence sometime between May 19, 1963-May 31, 1966 have been tested for CF antibody to the "O" agent. 150 (40%) of the children experienced at least one rotavirus infection; 11 had a second infection and one a third infection. For the period from May 22, 1966-May 21, 1969 65 (36%) of 182 children (some overlap with previous period) experienced at least one rotavirus infection, with 6 having a second infection. We will attempt to propagate selected rotavirus positive specimens in tissue culture by direct isolation or genetic reassortment in order to serotype them by recently developed techniques such as hybridization with single gene substitution reassortants or split phase immunoassay. This takes on increased importance since the natural history of strains from asymptomatic infections, which will likely be found in the longitudinal study, needs to be studied.</p>		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00334-04 LID
INACTIVE 1986

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Development of Monoclonal Antibodies to Rotavirus Proteins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Karen Midthun, M.D. Medical Staff Fellow LID, NIAID

Others: Albert Z. Kapikian, M.D. Head, Epid. Section LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20982

TOTAL MAN-YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In order to devise a practical, quick assay for serotyping human rotavirus isolates, repeated attempts at isolating monoclonal antibodies directed at the major neutralization protein of human rotaviruses have been made. In the past, monoclonal antibodies directed at the outer capsid proteins VP₂, the hemagglutinin, and VP₇, the major neutralization protein, of RRV were isolated. Screening by hemagglutination-inhibition assay was of key importance in identifying monoclonal antibodies directed at the outer capsid proteins of RRV. In order to isolate monoclonal antibodies directed at the major neutralization proteins of human serotypes 1, 2, and 4, Balb/C mice were immunized with human rotavirus x RRV reassortants which had human serotype specificity but contained the 4th gene (the hemagglutinin VP₃) and also the remaining genes from RRV. The mice were immunized approximately 3-4 times over a period of 2-4 months with partially purified virus. The fusion ratio was 10 spleen cells per NS-1 myeloma cell.

Screening of several fusions by hemagglutination-inhibition identified some monoclonal antibodies which inhibited hemagglutination of the reassortant rotavirus and all of these monoclonal antibodies were directed at the RRV 4th gene product, VP₃; none were directed at the human rotavirus VP₇ protein. A screening test involving neutralization of virus in 96 well tissue culture plates has been developed and will be used to screen for neutralizing antibodies to the different serotypes.

In addition, attempts are being made to develop an ELISA test which would enable serotyping of serotype 1 and 3 viruses by using monoclonal antibodies directed at the major neutralization protein of Wa (kindly supplied by Dr. Harry Greenberg) and at the major neutralization protein of RRV (which had been isolated in the past).

INACTIVE During 1985-86

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00335-05 LID
PERIOD COVERED October 1, 1985 to September 31, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Rotavirus Reassortants: Genetics and Use in Rotavirus Vaccines		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Karen Midthun, M.D.	Medical Staff Fellow LID, NIAID
Others:	Yasutaka Hoshino, D.V.M.	Visiting Scientist LID, NIAID
	Jorge Flores, M.D.	Visiting Scientist LID, NIAID
	Roger Glass, M.D.	Medical Officer LID, NIAID
	Albert Z. Kapikian, M.D.	Head, Epidemiology Sec. LID, NIAID
	Robert M. Chanock, M.D.	Chief LID, NIAID
COOPERATING UNITS (if any) <div style="text-align: center; padding: 10px;">Flow Laboratories, Inc. (L. Potash)</div>		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Epidemiology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS:	.2	PROFESSIONAL: .1 OTHER: .1
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> A practical application of single human rotavirus gene substitution reassortants is found in the study of genetic relatedness of the genes coding for VP₇ of human rotaviruses belonging to serotypes 1 through 4. Double-stranded (ds) genomic RNAs of human rotaviruses belonging to serotypes 1-4 were hybridized to single stranded (ss) mRNA probes derived from human-bovine rotavirus reassortants containing only the VP₇ gene of their human rotavirus parent. Bovine rotavirus genes do not hybridize to the corresponding genes of human rotaviruses and thus hybridization can only occur between the human VP7 gene in the labelled probe and the VP7 gene present in the human rotavirus under study. A high degree of homology was demonstrated between the VP₇ genes of a) strain D and other serotype 1 human rotaviruses, b) strain DS-1 and other serotype 2 human rotaviruses, c) strain P and other serotype 3 human human rotaviruses; and d) strain ST3 and other serotype 4 human rotaviruses. However, hybrid bands could not be demonstrated between the VP₇ genes of D, DS-1, P, or ST3 and human rotaviruses belonging to a different serotype. This technique was also used to examine RNA extracted from stools of children hospitalized with rotavirus diarrhea. All five viruses with a "short" RNA pattern shared homology with the DS-1 strain VP₇ gene; two of them had been previously adapted to tissue culture and shown to be serotype 2 strains by neutralization. Of the remaining 10 viruses, with "long" RNA patterns, two hybridized to the D strain VP7 gene, six hybridized to the P strain VP7 gene, and two hybridized to the ST3 strain VP7 gene. Hybridization using single human rotavirus gene substitution reassortants as probes may provide an alternate way of serotyping field isolates and would circumvent a need for tissue culture adaption. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00338-05 LID
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Molecular Cloning and Sequencing of Rotavirus Genes		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: Jorge Flores, M.D. Others: Kim Green, Ph.D.	Visiting Scientist Staff Fellow	LID, NIAID LID, NIAID
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Epidemiology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 1.1	PROFESSIONAL: 0.7	OTHER: 0.4
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> We have obtained and analyzed cDNA clones from several genes of various rotavirus strains. Single or double stranded RNAs were used for cDNA synthesis by reverse transcriptase after either polyadenylation and oligo (dT) priming or direct priming with synthetic oligonucleotides corresponding to the known end sequences of the genes. Double stranded cDNA molecules were inserted into plasmid vectors by either d.C./d.G. tailing or direct blunt end ligation. In many cases the RNA templates used for reverse transcription consisted of individually isolated genes (by preparative PAGE); otherwise identification of the gene segment present in each clone was established by Northern blot, hybridization or colony hybridization with cDNA probes from clones of known origin. </p> <p> Clones are now available that include copies of the VP7 gene of the animal rotavirus strains NCDV and UK (bovine), OSU (porcine), and RRV (simian) and the human rotavirus strains Wa, DS-1, P, M37 and ST3. Also, VP3 gene clones from RRV and NCDV have been obtained. Direct dideoxy sequencing of either mRNAs or cloned cDNAs has been performed for the VP7 gene of a number of strains by using sequence-specific oligonucleotide synthetic primers. In addition to the OSU and NCDV VP7 glycoprotein gene sequences, the complete sequence of the RRV VP7 glycoprotein gene has been determined as well as most of the corresponding sequence of the human strains D (serotype 1), DS-1 (serotype 2), P (serotype 3) and ST3 and VA70 (both serotype 4). </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00339-05 LID
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>Isolation and Serotypic Characterization of Human and Animal Rotaviruses</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Yasutaka Hoshino, D.V.M. Visiting Scientist LID, NIAID		
COOPERATING UNITS (if any) The Children's Hospital of Philadelphia, PA (Dr. H. Fred Clark); James N. Gamble Institute of Medical Research, Cincinnati, OH (Richard L. Ward).		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Epidemiology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 0.4	PROFESSIONAL: 0.1	OTHER: 0.3
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>A naturally occurring intertypic porcine rotavirus strain SB-1A was analyzed genetically and serologically. It was shown that this isolate was serotype 4 based on its VP7 and serotype 5 based on its VP3. By analyzing single VP3 gene substitution reassortants, we confirmed that VP3 was as potent in inducing neutralizing antibodies as VP7. The fourth genome segment of porcine rotavirus strains SB-1A and OSU and canine rotavirus strain CU-1 was shown to code for viral hemagglutinin.</p> <p>Human rotavirus strain WI-61 was found to be distinct serotypically from the four known human serotypes.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00340-05 LID
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Experimental Studies in Animals with Various Rotaviruses and Their Reassortants		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Yasutaka Hoshino, D.V.M. Visiting Scientist LID, NIAID		
COOPERATING UNITS (if any) <div style="text-align: center;">Ohio Agricultural Research and Development Center, Wooster, Ohio (Dr. Linda Saif)</div>		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Epidemiology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 0.2	PROFESSIONAL: 0.1	OTHER: 0.1
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> Porcine OSU and Gottfried rotaviruses are distinct by neutralization and thus both their VP3 and VP7 outer capsid proteins are unrelated antigenically. In order to study these neutralization proteins a single gene substitution OSU x Gottfried porcine rotavirus reassortant (11-1) was generated by gene reassortment. This reassortant derived 10 genes (including the fourth gene encoding VP3) from the OSU strain (serotype 5) and only the ninth gene (encoding the other major neutralization glycoprotein [VP7]) from the Gottfried strain (serotype 4). Sera obtained from gnotobiotic piglets three weeks after initial oral administration of this reassortant contained high levels of neutralizing antibodies not only to Gottfried, mediated by VP7, but also to OSU, mediated by VP3. This established that VP3 is as effective an immunogen as VP7 in inducing neutralizing antibodies during experimental intestinal infection. When a group of piglets initially infected with the reassortant was challenged three weeks later with virulent Gottfried virus, complete protection was observed as indicated by failure of symptoms to develop and failure to detect virus shedding. Following challenge of another group of piglets (previously infected with the reassortant) with virulent OSU virus, complete protection against diarrhea was also observed and virus shedding was delayed in onset and decreased in duration. These observations indicate that: (i) VP3 antibodies induced during initial rotavirus infection confer resistance to disease produced by virulent rotavirus and (ii) a reassortant rotavirus bearing VP3 and VP7 neutralization antigens derived from separate rotaviruses which are distinct by neutralization induces immunity to both parental viruses. </p>		
8-77		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00341-05 LID
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Evaluation of Experimental Rhesus Rotavirus Vaccine in Infants and Children		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Albert Z. Kapikian, M.D. Head, Epidemiology Section LID, NIAID		
Others: Jorge Flores, M.D. Visiting Scientist LID, NIAID Roger Glass, M.D. Medical Officer LID, NIAID Karen Midthun, M.D. Senior Staff Fellow LID, NIAID Yasutaka Hoshino, D.V.M. Visiting Scientist LID, NIAID Robert M. Chanock, M.D. Chief, LID LID, NIAID		
COOPERATING UNITS (if any) Flow Laboratories; U.Md.; Johns Hopkins U.; U. Rochester; Vanderbilt U.; Marshall U.; U. Tempere, Finland; U. Umea, Sweden; Nat. Inst. Dermatology, Venezuela; Johns Hopkins U.; King Edward Med.Coll., Pakistan; Agency Int. Development.		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Epidemiology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS:	4.5	PROFESSIONAL: 1.1 OTHER: 3.4
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Rotaviruses have emerged as the single most important etiologic agents of severe diarrhea of infants and young children in both developed and developing countries. Thus, the need for an effective rotavirus vaccine is clear. The goal of such a vaccine is to prevent severe rotavirus diarrhea during the first 2 years of life when this disease is most serious. An animal rotavirus strain, rhesus rotavirus (RRV), is under intensive study in LID as a vaccine candidate. This simian rotavirus has not been recovered under natural conditions from man and is thus not a virus of the human heritage. Although the genes of RRV exhibit significant divergence in sequence from the corresponding genes of human rotaviruses, this simian rotavirus is similar if not identical to human rotavirus type 3 when tested by neutralization. The RRV vaccine was found to be satisfactorily antigenic as well as non-reactogenic in adults and children. However, in several pediatric studies a transient febrile response and/or loose stools occurred when 6-12 month old infants were fed 10^5 PFU of virus. At a dose of 10^4 PFU the RRV retained its antigenicity in infants less than 6 months of age but did not cause significant reactions. Analysis of prevaccination serum RRV neutralizing antibody titers in several populations, some in which reactions occurred and some in which reactions did not occur, suggested that the reactions might be related to the absence of serum antibody to RRV. Thus, prevaccination serum RRV antibodies may modify clinical reactions to RRV vaccine without significantly affecting infectivity or antigenicity. Following the identification of a safe antigenic dose of vaccine several phase two double blind field trials were initiated with various collaborators in the U.S. and other parts of the world in order to assess the efficacy of this vaccine in infants under 6 months of age.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00342-05 LID
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Studies of Gastroenteritis Viruses by Electron Microscopy		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Albert Z. Kapikian, M.D. Head, Epid. Sect. LID, NIAID		
COOPERATING UNITS (if any) LID/NIAID (J. Ticehurst, R. Purcell); LVD/NIAID (G. Kotwal)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Epidemiology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 0.2	PROFESSIONAL: 0.1	OTHER: 0.1
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The electron microscope (EM) has been a mainstay for study of fastidious gastroenteritis viruses. Despite the development of 2nd and 3rd generation tests, EM remains an indispensable tool: (1) as the "supreme court" when newer tests yield variable results; (2) in the search for new agents of viral gastroenteritis, (3) for visualizing the site of attachment of antibody to the virion in antigen-antibody reactions; (4) for serologic studies; (5) for direct visualization and fine structure characterization of the morphology of virus particles; and (6) for studying specimens derived from individuals with diseases of unknown etiology (such as non-A, non-B hepatitis) by immune electron microscopy. A new technique was introduced last year - solid phase immune electron microscopy - which was used successfully for the rapid serotyping of human and animal rotaviruses. In addition, the technique appeared to be more sensitive than conventional immune electron microscopy for rotavirus detection. This past year another new technique was introduced -- colloidal gold conjugated to protein A -- as a means of facilitating the recognition of the specific site of antigen-antibody interaction. Finally, since over 50% of the episodes of pediatric diarrhea are without known etiology, EM should continue to prove to be a valuable tool in the search for such agents. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00343-05 LID
PERIOD COVERED <u>October 1, 1985 to September 30, 1986</u>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>The Role of Norwalk-Like 27nm Virus Particles in Viral Gastroenteritis</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Karen Midthun, M.D.	Medical Staff Fellow LID, NIAID
Others:	Albert Z. Kapikian, M.D.	Head, Epid. Section LID, NIAID
COOPERATING UNITS (if any)		
LAB/BRANCH		
<u>Laboratory of Infectious Diseases</u>		
SECTION		
<u>Epidemiology Section</u>		
INSTITUTE AND LOCATION		
<u>NIAID, NIH, Bethesda, Maryland 20892</u>		
TOTAL MAN-YEARS:	0.2	PROFESSIONAL: 0.2
		OTHER: 0
CHECK APPROPRIATE BOX(ES)		
<input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)		
<p>The Marin County agent is a 27nm virus-like particle which was associated with two separate outbreaks of nonbacterial gastroenteritis in northern California in 1978 by L. Oshiro. The agent is morphologically similar to, but serologically distinct from the Norwalk, Hawaii and Snow Mountain agents as assessed by immune electron microscopy (IEM) or solid phase radioimmunoassay (RIA) antibody blocking assay. One ml of a safety tested, bacteria-free filtrate prepared from a stool specimen from one of the individuals ill during the original Marin County outbreak was administered orally to seventeen adult volunteers. None of these individuals developed definite clinical illness. Two additional volunteers later received a 20ml inoculum in order to enhance the probability of inducing illness by increasing the dose. One of these volunteers developed a gastrointestinal illness characterized by nausea, vomiting, diarrhea and malaise. Interestingly, this illness started five days after administration of the fecal filtrate and lasted 36-48 hours. Examination by IEM of several diarrheal stool specimens from this volunteer demonstrated a large number of 27nm particles. These particles were shown to be identical to the Marin County agent in IEM studies using acute and convalescent sera from the original outbreak. A preliminary survey of a series of gastroenteritis outbreaks using a recently developed RIA failed to implicate the Marin County agent as an important cause of epidemic gastroenteritis.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00346-05 LID
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>Genetic Characterization of Rotavirus by Hybridization Techniques</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Jorge Flores, M.D.	Visiting Scientist LID, NIAID
Others:	Kim Green, Ph.D. Yasutaka Hoshino, D.V.M. Karen Midthun, M.D.	Staff Fellow LID, NIAID Visiting Scientist LID, NIAID Medical Staff Fellow LID, NIAID
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Epidemiology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS:	0.8	PROFESSIONAL: 0.4 OTHER: 0.4
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) <p>Rotaviruses undergo genetic variation by one of two mechanisms: a) accumulation of successive mutations within specific genes (genetic drift); and b) gene reassortment (genetic shift) that results in the appearance of rotavirus strains with a constellation of genes which are derived from two or more distinct rotaviruses. The relative importance of these two mechanisms (genetic drift or shift) in the generation of new strains is not known. Partial sequence analysis of nosocomial rotavirus strains recovered from neonates with asymptomatic infection suggests that the rotavirus genome does not undergo a high rate of spontaneous mutation; on the other hand, rotavirus strains have been identified that appear to have arisen by gene reassortment.</p> <p>In studies in which the RNAs of rotaviruses isolated from asymptomatic newborn infants ("nursery strains") were compared by RNA-RNA hybridization with strains from ill infants a marked difference in the sequence of the fourth gene was detected. Whereas, the fourth gene is highly conserved among the nursery strains, it differs significantly from the corresponding conserved gene of strains isolated from ill infants. These differences in the fourth gene occur independent of serotype (VP7), subgroup specificity (VP6) or the remaining genes that code for structural or non-structural proteins. It is likely that the reduced virulence of the "nursery rotavirus strains" is determined entirely or in part by the unique conserved sequences of the fourth gene of these viruses. This relationship has important implications for the future design of safe, effective rotavirus vaccines.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00410-02 LID TERMINATED 1986
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Binding of Rotavirus to Cell Surface Receptors		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <div style="display: flex; justify-content: space-between;"> PI: Jon Askaa, D.V.M. Visiting Fellow LID, NIAID </div>		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Epidemiology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS:	0.1	PROFESSIONAL: 0.1 OTHER: 0.0
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input type="checkbox"/> (b) Human tissues </div> <div> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> In an attempt to characterize the nature of the receptors on cell surfaces and of the viral protein involved in this initial interaction between rotavirus and cells, several approaches have been followed. (1) Enzymatic treatment of human type O erythrocytes before utilization in hemagglutination assay has resulted in the separation of rhesus and bovine (NCDV strain) rotaviruses into one group and two avian rotavirus isolates into another group with respect to their ability to agglutinate these enzymatically treated erythrocytes. (2) Non-hemagglutinating human rotaviruses have been shown to bind to human erythrocytes in a modified radioimmunoassay using the erythrocytes as solid phase. (3) Labeled rotavirus has been shown to react with membrane proteins isolated from both the microvilli of enterocytes of the small intestine of pigs as well as from human erythrocytes. (4) Rotavirus was demonstrated to bind to glycosphingolipids in a thin layer chromatography system. (5) The viral protein involved in the initial binding to MA 104 cells has in preliminary experiments been found to have a molecular weight of approximately 20,000 daltons. Attempts to produce monoclonal antibodies against membrane proteins isolated from enterocytes as well as from erythrocytes have been carried out. Production of monoclonal antiidiotypic antibodies against the protein encoded by gene 4 or gene 9 of rhesus rotavirus has also been attempted. </p> <p style="text-align: center; margin-top: 20px;"><u>TERMINATED 1986</u></p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00 461-01 LID TERMINATED 1986
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less; Title must fit on one line between the borders.) Serial Passages of Bovine and Rhesus Rotaviruses		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Jon Askaa, D.V.M. Visiting Fellow LID, NIAID		
Others: Mario Gorziglia, Ph.D. Visiting Fellow LID, NIAID Karen Midthun, M.D. Med. Staff Fellow LID, NIAID Yasutaka Hoshino, D.V.M. Visiting Associate LID, NIAID Jorge Flores, M.D. Visiting Scientist LID, NIAID		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Epidemiology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 0.1	PROFESSIONAL: 0.1	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Genomic rearrangement of bovine rotavirus (NCDV strain) has been found following serial passage in cell culture at initial high multiplicity of infection. Plaque purified virus from passage 14 contained dsRNA with changes in electrophoretic pattern. Gene segment 5 could not be detected while a new band migrating between RNA segments 1 and 2 appeared. In a hybridization experiment homology was observed between gene segment 5 and the new band. Differences in the production of viral proteins between the two viruses could not be detected.</p> <p>Attempts to demonstrate (1) interference between these viruses and (2) genomic rearrangement in other serotypes are in progress.</p> <p style="text-align: center;"><u>TERMINATED 1986</u></p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00462-02 LID
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Rotavirus Vaccine Field Trial in Venezuela		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: Jorge Flores, M.D.	Visiting Scientist	LID, NIAID
Others: Albert Z. Kapikian, M.D.	Section Head	LID, NIAID
COOPERATING UNITS (if any) <div style="text-align: center;"> Instituto Nacional de Biomedicine, Caracas, Venezuela (Dr. Perez-Schael, Dr. Gonzalez, Dr. Daoud) </div>		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Epidemiology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 0.5	PROFESSIONAL: 0.2	OTHER: 0.3
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> We have continued our investigations in Venezuela of the potential usefulness of the rhesus rotavirus MMU18006 strain vaccine candidate. This virus is a simian rotavirus strain derived from a stool of a 3.5 month old rhesus monkey with acute diarrhea. In phase I and II double-blind studies, the vaccine was administered to 120 infants (1-10 months old). A similar number of infants received placebo. The infants were examined daily for presence of side reactions. Rectal temperature was taken twice a day by medical personnel and stool specimens were collected daily and analyzed for rotavirus shedding. Significant reactions were not observed in the infants who received the vaccine when compared to the placebo group. Serological assays performed for rotavirus antibody included complement-fixation, plaque reduction neutralization and tube neutralization. Overall, 69.3 of the vaccinated children developed a seroresponse. Phase I studies in newborn infants have also been initiated. Efficacy studies are being carried out by continuous surveillance of the 1-10 infants in the vaccine and placebo groups. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00463-01 LID TERMINATED 1986
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Oligonucleotide Probes for Detection and Identification of Rotavirus Serotypes		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: Roger Glass, M.D. Others: Jorge Flores, M.D. Yasutaka Hoshino, D.V.M. Jerry Keith, Ph.D.	Medical Officer Visiting Scientist Visiting Associate Section Chief	LID, NIAID LID, NIAID LID, NIAID Rocky Mtn Lab, NIAID
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Epidemiology Section		
INSTITUTE AND LOCATION NIAID/NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: <div style="display: flex; justify-content: space-between; width: 100%;"> 1.1 0.5 0.6 </div>	PROFESSIONAL:	OTHER:
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> Labelled oligonucleotides are being used extensively as genetic probes because they are relatively easy to make and under ideal conditions, can discriminate by hybridization between gene sequences that differ by a single base pair. The recent proliferation of sequences for the neutralization gene of different strains of rotaviruses encouraged us to prepare oligonucleotide probes to areas common to all serotypes of rotavirus as well as to areas demonstrating great serotypic diversity. We have tested these probes for their sensitivity and specificity in detecting rotavirus in stool specimens and in serotyping those specimens found to be positive. We have also compared the sensitivity of short oligonucleotide probes with larger single stranded RNA transcripts prepared according to procedures developed previously in this laboratory. Radiolabeled oligonucleotide probes appear to be about as sensitive as the ELISA test in detecting rotavirus in stool specimens and are significantly less sensitive than ssRNA transcript probes. The level of sensitivity and specificity are reduced when biotinylated oligonucleotide probes are used. Serotyping will be examined by hybridizing oligo probes to Northern blots of viral RNA since neither specificity nor sensitivity were adequate using dotted stool RNAs. </p> <p style="text-align: center; margin-top: 20px;"> <u>TERMINATED 1986</u> </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00448-01 LID TERMINATED 1986
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Rotavirus Vaccine Trial in Umea, Sweden		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> PI: Roger Glass, M.D. Albert Z. Kapikian, M.D. </div> <div style="width: 50%;"> Medical Officer LID, NIAID Head, Epidemiology Sect. LID NIAID </div> </div>		
COOPERATING UNITS (if any) University of Umea, Umea, Sweden (Dr. Goran Wadell, Dr. Leif Gothefors)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Epidemiology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS:	0.0	PROFESSIONAL: 0.0
		OTHER: 0.0
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>A field trial of the rhesus rotavirus (RRV) vaccine was begun in Umea, Sweden in January 1985 to examine the efficacy of the vaccine given at a dose of 10^8 pfu. 106 infants 4-12 months of age were given placebo or vaccine with a bicarbonate-citrate buffer at the start of the rotavirus season. These individuals will be followed actively for rotavirus diarrhea and serologic responses for 18 months.</p> <p>In the first month of followup, vaccinees had a significantly greater number of low grade fevers and loose stools than placebo recipients. Consequently, 2 phase I trials are being planned before recruitment of more infants into the trial continues. In one trial, reactogenicity of a lower dose of vaccine and the importance of the buffer will be examined by giving groups of 15-20 infants aged 4-12 months a lower dose of the vaccine with the bicarbonate-citrate buffer and without buffer. A third group will receive placebo alone. In the second phase I trial, immunogenicity and reactogenicity of the lower dose vaccine will be examined among infants 1 month of age.</p> <p>In the field trial, about 40 infants have had diarrhea during the first rotavirus season so this trial could potentially be the first to establish efficacy of the RRV vaccine.</p>		
CONSOLIDATED WITH Z01 AI 00341-05 <u>TERMINATED 1985-86</u>		
8-86		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00450-02 LID
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Sequence of the Fourth Rotavirus Gene and Its Role in Virulence		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: Mario Gorziglia, Ph.D. Others: Yasutaka Hoshino, D.V.M. Albert Z. Kapikian, M.D. Alicia Buckler-White Robert M. Chanock, M.D.	Visiting Fellow Visiting Scientist Head, Epid. Section Staff Fellow Chief, LID	LID, NIAID LID, NIAID LID, NIAID LID, NIAID LID, NIAID
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Epidemiology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 1.5	PROFESSIONAL: 1.1	OTHER: 0.4
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The fourth gene of rotavirus strains recovered from outbreaks of asymptomatic infection in newborn nurseries exhibits a marked conservation of sequence. Similarly, the fourth gene of virulent rotaviruses exhibits conservation of sequence but this conserved sequence differs from that of the asymptomatic strains. This gene 4 sequence dimorphism was studied by sequence analysis of the region of the fourth gene that codes for the VP8 protein, downstream cleavage sites and the N terminus of VP5. The human rotaviruses exhibit many similarities in this region of their genome, including identical N-terminal amino acid sequences, conservation of arginine at the two trypsin cleavage sites and the position of a cysteine residue. Alignment of amino acid sequences of asymptomatic and virulent human rotavirus strains indicates a high degree of homology (96% or more) among the asymptomatic viruses (serotypes 1, 2, 3 and 4), while homology between asymptomatic strains and virulent viruses is considerably less (68-72%). A high degree of conservation of amino acid sequence (92-97%) is also observed among 3 of the virulent strains (serotypes 1, 3 and 4). At 48 positions in the protein sequence of VP8, the cleavage region and the N terminus of VP5 an amino acid is conserved among asymptomatic rotaviruses, while a different amino acid is conserved among virulent rotaviruses. Notably, three of these differences are located within the short 6 amino acid cleavage region between VP8 and VP5. It is possible that some or all of this sequence dimorphism may be responsible for the difference in virulence between these two groups of human rotaviruses. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00451-02 LID
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Use of Gene Reassortment to Study Rotavirus Gene Products		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Yasutaka Hoshino, D.V.M. Visiting Scientist LID, NIAID		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Epidemiology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 1.2	PROFESSIONAL: 0.5	OTHER: 0.7
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The genome of rotaviruses, members of the reoviridae family, consists of 11 discrete segments (genes) of dsRNA. Its unusual genome structure (segmentation) and the relative ease of generation of reassortants <u>in vitro</u> have provided a unique opportunity to gain insight into genetic and molecular mechanisms of virus-host interactions through mapping of biological function of specific genes. During the past year, efforts were made to generate single gene substitution human x human rotavirus reassortants which would be potential human rotavirus vaccines. In addition, antigenic analysis of VP3 (the fourth gene product) and VP7 (the eighth or ninth gene product) of rotavirus was performed.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00452-01 LID TERMINATED 1986
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Characterization of Rotaviruses From Asymptomatic Human Neonatal Infections		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
P.I.:	Yasutaka Hoshino, D.V.M.	Visiting Associate LID, NIAID
Others:	Jorge Flores, M.D.	Visiting Scientist LID, NIAID
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Epidemiology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.0	0.0	0.0
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> Nineteen rotavirus strains derived from asymptomatic neonates (seven from England, five from Australia, two from Venezuela, and five from Sweden) were successfully cultivated in primary African green monkey kidney cell cultures, serotyped by plaque reduction neutralization (PRN) assay, subgrouped by indirect enzyme-linked immunosorbent assay, and electrophoretotyped by polyacrylamide gel electrophoresis. All 19 strains were shown to fall into one of the four known human serotypes; serotype 1 (all Venezuelan strains), serotype 2 (all Swedish strains), serotype 3 (all Australian strains), or serotype 4 (all English strains). Hyperimmune guinea pig antiserum raised against the Venezuelan strain (M37) neutralized not only serotype 1 (strain Wa) but also serotype 4 (strain St. Thomas no. 3) viruses to a similar degree. The English, Australian, and Venezuelan isolates were found to belong to subgroup 2, and the Swedish strains were subgroup 1 viruses. </p> <p style="text-align: center; margin-top: 100px;"> <u>TERMINATED 1985-86</u> </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00453-01 LID TERMINATED 1986
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Vaccinia Recombinant Containing Bovine Rotavirus Glycoprotein Gene		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Osamu Nakagomi, M.D., Ph.D. Visiting Fellow LID, NIAID		
Others: Jorge Flores, M.D. Visiting Scientist LID, NIAID Toyoko Nakagomi, M.D., Ph.D. Guest Worker LID, NIAID Yasutaka Hoshino, D.V.M. Visiting Associate LID, NIAID Robert M. Chanock, M.D. Chief, LID LID, NIAID Albert Z. Kapikian, M.D. Head, Epid. Sect. LID, NIAID		
COOPERATING UNITS (if any) LVD, NIAID (Dr. Bernard Moss, Dr. Geoffrey Smith)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Epidemiology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 0	PROFESSIONAL: 0	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> We constructed a vaccinia virus recombinant which expressed bovine rotavirus glycoprotein VP7 (the major neutralization protein) by inserting a cDNA copy of the 9th gene from NCDV into the TK gene of vaccinia virus. This recombinant virus expressed a polypeptide of approximately 35,000 dalton which migrated closely with VP7 of NCDV. We vaccinated two rabbits with this recombinant virus intradermally and observed their response by various serological methods. Immunofluorescent and plaque reduction neutralization tests showed a significant increase in titer following vaccination although relatively high levels of pre-existing antibody to rotavirus made proper interpretation difficult. </p> <p> This preliminary experiment demonstrated the necessity of using animals lacking rotaviruses antibody in further animal experiments to determine the antigenicity of this recombinant. We screened sera from cotton rats, mice, hamsters, guinea pigs and rabbits by neutralization assay. Although many of the animals had rotavirus antibody, it appeared that, if a sufficient number of mice, hamsters, or guinea pigs were used, a small proportion should have little, if any antibody. Thus, mice and hamsters have been inoculated with the recombinant vaccine virus to study their serological response to the NCDV VP7 component of the vaccinia-rotavirus VP7 recombinant. </p> <p style="text-align: center; margin-top: 20px;"> <u>TERMINATED 1985-86</u> </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00454-01 LID TERMINATED 1986
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Expression of Bovine Rotavirus Glycoprotein Gene in Mammalian Cells		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Osamu Nakagomi, M.D., Ph.D.	Visiting Fellow LID, NIAID
Others:	Toyoko Nakagomi, M.D., Ph.D. Jorge Flores, M.D.	Guest Worker LID, NIAID Visiting Scientist LID, NIAID
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Epidemiology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>A full-length complementary DNA copy of the 9th gene of the bovine rotavirus NCDV strain which codes for a glycoprotein that induces neutralizing antibody was cloned into the late region of pSV2330, a hybrid expression vector that includes pBR322 plasmid DNA sequences, the simian virus 40 (SV40) early region and SV40 late region promoters, splice sequences, polyadenylation sequences and transcription termination sites. A near full-size cDNA copy of NCDV gene 9 which lacks the first but not the 2nd translation initiation codon is also ready to be cloned into pSV2330. This pSV2330 - monkey kidney cell system has proven by other researchers in this laboratory to be useful for studying influenza viral proteins that must be post translationally modified to achieve their biological activity. Partly based on their experience, we will first examine the antigenicity of the recombinant protein product by monoclonal antibodies and then ask whether either glycosylation or N-terminal hydrophobic region plays a role in its localization within the transfected cells. The findings which will be obtained in this study should broaden our understanding of rotavirus infection.</p>		
<u>TERMINATED 1985-86</u>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00455-01 LID TERMINATED 1986
PERIOD COVERED October 1, 1985 through September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Expression of Rotavirus Neutralization Protein In Bacteria		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
P.I.: Toyoko Nakagomi, M.D., Ph.D. Guest Worker LID, NIAID		
Others: Osamu Nakagomi, Ph.D. Visiting Fellow LID, NIAID Jorge Flores, M.D. Visiting Scientist LID, NIAID Jon Askaa, D.V.M. Visiting Fellow LID, NIAID		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Epidemiology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 0	PROFESSIONAL: 0	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> We have attempted to express rotavirus antigen in <u>E. coli</u> as a means of developing an effective and safe rotavirus vaccine. If such an antigen is located on the bacterial surface it may stimulate local immunity by colonizing the small intestine. Toward this goal, we have utilized an open reading frame (ORF) expression vector (pORF2) which may direct the expression of rotavirus gene segments. The insertion of rotavirus cDNA sequences in this vector may allow the expression of hybrid proteins which could be transported to the cell surface. </p> <p> When we cloned various sets of Sau 3A partial digests of cDNA of the NCDV or RRV gene encoding VP7 into pORF2, only a few of these constructs expressed recombinant molecules, although the in-frame insertion of the gene segments into pORF2 had been achieved. The highest levels of expression (up to 14% of <u>E. coli</u> protein) were achieved with the shorter segments, however the resulting hybrid proteins tested by immunoprecipitation were not recognized by either polyclonal or monoclonal antisera. When we cloned larger fragments of the NCDV VP7 gene the level of expression was not high enough to allow further studies with this system. We also made constructs in which a λ PL promoter fragment was introduced 5' upstream of the fusion genes instead of ompF promoter originally provided by the pORF2 vector. The level of expression achieved with this stronger promoter was, however, not significantly increased. </p> <p> The PL promoter has also been used to attempt expression of defined segments of the NCDV gene 9 (VP7 gene). One such construct (carrying 822bp coding sequence) directed the expression of a protein of $\pm 28,000$ daltons when transformed into <u>E. coli</u>. Immunological examination of this protein is in progress. </p>		
TERMINATED 1985-86		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00456-01 LID TERMINATED 1986
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Assay of Coproantibodies to Rotavirus		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Rebecca Tominack, M.D. Medical Staff Fellow	LID, NIAID
Others:	Karen Midthun, M.D. Medical Staff Fellow	LID, NIAID
	Albert Kapikian, M.D. Head, Epid. Section	LID, NIAID
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Epidemiology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.0	0.0	0.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) <p> We are attempting to develop a solid phase ELISA system for the detection and quantitation of immunoglobulins in human stool specimens directed against rotavirus. Major efforts to date have been directed at establishing reliable and optimal procedures and reagents for each step in this multi-layered "sandwich" assay. The basic test system is: 1) precoat 96 well plates with hyperimmune goat 930 rotavirus antiserum as capture antibody 2) add rotavirus antigen (3) add test specimens of serum or stool containing antibodies to rotavirus (4) add antibodies to human immunoglobulin conjugated to peroxidase and (5) add specific substrate for peroxidase which causes development of color that can be read as optical density units. To date several difficulties have been encountered. The most confounding difficulty has been the long unrecognized intermittent partial/total failure of the conjugate. One other problem is that of high background color due to non-specific interactions with the precoat and several modifications will be explored to overcome this difficulty including use of purified rotavirus antigen, treatment to block "nonspecific" sites, change in the order of the ELISA sandwich layers. </p> <p style="text-align: center; margin-top: 20px;"> <u>TERMINATED 1985-86</u> </p> <p style="text-align: center; margin-top: 20px;"> 8-94 </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00478-01 LID
PERIOD COVERED <u>October 1, 1985 to September 30, 1986</u>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>Development of Adenovirus Expression Vectors</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: Jorge Flores, M.D. Others: Harold S. Ginsberg, M.D. Kim Y. Green, Ph.D.	Visiting Scientist IPA Staff Fellow	LID, NIAID LID, NIAID LID, NIAID
COOPERATING UNITS (if any)		
LAB/BRANCH <u>Laboratory of Infectious Diseases</u>		
SECTION <u>Epidemiology Section</u>		
INSTITUTE AND LOCATION <u>NIAID, NIH, Bethesda, Maryland 20892</u>		
TOTAL MAN-YEARS: 0.6	PROFESSIONAL: 0.4	OTHER: 0.2
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> Engineering the adenovirus genome to allow the insertion of foreign genes may yield infectious recombinant viruses that express the foreign genes. Such recombinants could potentially be used as vaccines. We have made some progress in this approach including the growth and purification of adenovirus types 4 and 7 from large suspensions, the isolation and restriction mapping of their genomes and the cloning in plasmids of the E3 region and adjacent areas of the adenovirus 4 genome. The right hand end of the adenovirus 4 genome (map units 83-100) has also been cloned. The ends of the available clones have been sequenced and primers have been synthesized to localize the major late promoter and the tripartite leader sequences. The VP7 glycoprotein gene of several rotavirus strains and part of the fourth rotavirus gene (the area which encodes VP8 and a portion of VP5) have been cloned in pBR322 or in the polylinker area of pUC vectors to facilitate their transfer into the adenovirus genome. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00479-01 LID
PERIOD COVERED <u>October 1, 1985 to September 30, 1986</u>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>Development of Monoclonal Antibodies to Proteins of Rotavirus</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Yasutaka Hoshino, D.V.M. Visiting Scientist LID, NIAID		
COOPERATING UNITS (if any)		
LAB/BRANCH <u>Laboratory of Infectious Diseases</u>		
SECTION <u>Epidemiology Section</u>		
INSTITUTE AND LOCATION <u>NIAID, NIH, Bethesda, Maryland 20892</u>		
TOTAL MAN-YEARS: 0.7	PROFESSIONAL: 0.3	OTHER: 0.4
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> An equine rotavirus strain FI-14 was shown to belong to serotype 3 by neutralization assay. In addition, it was found to react with both subgroup I and subgroup II monoclonal antibodies by ELISA, thus representing the first rotavirus strain to have both subgroup specificities. Using hybridoma technology, we successfully produced monoclonal antibodies directed against the major inner capsid protein VP6 (the sixth gene product) of FI-14 virus. These monoclonal antibodies reacted specifically with either subgroup I or subgroup II rotaviruses thus demonstrating that the VP6 of FI-14 virus has both subgroup I and subgroup II-specific epitopes. Four additional monoclonal antibodies directed to the VP-6 of FI-14 demonstrated distinct reactivities by ELISA with a panel of 22 rotavirus strains derived from 11 different animal and avian species. Thus, at least six distinct epitopes were shown to exist on VP6 of FI-14 virus. When analyzed by radioimmunoprecipitation, the molecular weight of the FI-14 virus VP6 (subgroups I and II) was found to be larger (approximately 45K) than the VP6 (approximately 42K) of rhesus rotavirus MMU18006 (subgroup I) or the VP6 of human rotavirus Wa (subgroup II). By RNA-RNA hybridization analysis, the FI-14 virus was shown not to share significant homology with any of the four known human rotavirus serotypes. </p>		

12
13
14
15
16
17
18

19
20
21
22
23
24
25

LABORATORY OF MICROBIAL IMMUNITY
1986 Annual Report
Table of Contents

<u>Project</u>	<u>Z01 AI Number</u>		<u>Page</u>
Summary			9-1
00131-19		Mechanism of Hypersensitivity In Inbred Histocompatible Guinea Pigs--Stone	9-3
00134-24		Control of Immunoglobulin Syntheses In Mice--Asofsky	9-4
00136-14		Characterization and Differentiation of Thymic Lymphocytes--Fowlkes	9-5
00141-10		Immune Responses to Malaria--Asofsky	9-6
00143-17		Genetic Control of the Antibody Response To Microbial Antigens--Baker	9-7
00144-22		Regulation of the Antibody Response To Microbial Polysaccharide Antigens--Baker	9-8
00145-19		Mode of Action of Thymus-Derived (T) Suppressor and Amplifier Cells--Baker	9-9
00146-12		Immunological Studies of Components Isolated From Bacteria, Parasites, and Plants--Caldes	9-10
00153-09		In Vitro Responses of Human Peripheral Leukocytes--Chu	9-11
00423-03		Factor Mediated Regulation of B Cell Growth and Differentiation--Howard	9-12

10
 11
 12
 13
 14

PHS-NIH
SUMMARY REPORT

ANNUAL REPORT OF THE LABORATORY OF MICROBIAL IMMUNITY, NIAID
October 1, 1985 to September 30, 1986

Richard Asofsky, MD
Chief, Laboratory of Microbial Immunity

Memory B Cells Preferentially Activated By Bacterial Lipopolysaccharide

Polyclonal activation of B cells by bacterial lipopolysaccharide (LPS) results in greater numbers of B cells synthesizing and secreting IgM antibody specific for sheep erythrocytes, Type III pneumococcal polysaccharide (SSS-III) or heterologous LPS in mice previously given a single injection of a sub-immunogenic dose of these antigens-with or without complexing to a carrier- so as to generate antigen- specific memory cells. The kinetics for the expression of such antibody synthesis are similar to those for polyclonal activation of B cells by LPS. Thus, it would appear that the polyclonal activation of B cells by LPS involves the preferential activation of memory, rather than virgin, B cells capable of responding specifically to antigen. (P.J. Baker and M. Fauntleroy, LMI, NIAID).

Differentiation of Immature Thymocytes in vitro

Immature (precursor; Ly1 dull, Ly2⁻) thymocytes cultured in vitro with PMA and ionomycin differentiate into Ly2⁺ cells and cells which secrete small amounts of IL-2. Such cells lack surface TcR, although they contain mRNA for the α , β chains of this structure. If the same cells are cultivated in the presence of IL-1, IL2, IL3, and as yet unidentified factor(s) in supernatants of spleen cells cultured in the presence of concanavalin A, 100% T3⁺ cells develop by selection or differentiation. Some of these cells express a mature, Ly2⁺, L3T4⁺ phenotype. The cells express a surface ϵ structure complexed to T3. This finding is the first demonstration of ϵ chains at the protein level in the mouse. Although the ϵ chain is not characterized yet, the finding of a T3-(ϵ) complex suggests this structure is an "immature" antigen receptor. (Drs. Fowlkes, Pardoll, (LI), Schwatz (LI), Lew (LIG), Kruisbeek (NCI))

Dissociation of inflammation and Demyelination in EAE

Experimental autoimmune encephalomyelitis (EAE) is a model for demyelinating diseases of man; in its chronic form in guinea pigs, it is the best model for multiple sclerosis (MS) both clinically and histologically. As in MS, there is a dichotomy between the histological evidence for EAE and the clinical manifestations. The significance of this anomaly is being sought by current cellular approaches. In dose-response, less than 150 micrograms of myelin basic protein induces severe clinical disease in strain 13 guinea pigs but no demyelination. The other side of the coin: in strain 2, severe inflammatory changes and demyelination can be elicited in clinically healthy sensitized animals. To determine whether the latter deficiency resides in either the sensitized cells or in the target organs, lymph node cells from sensitized strain 2 or 13 donors were transfused into (inherently histocompatible) 2/13 F1 hybrids susceptible to clinical disease. Whereas strain 13 cells easily

elicited clinical manifestations, very much larger numbers of strain 2 cells enhanced for T cell content by panning were needed, indicating a shortage of lymphocyte factor able to produce neural incapacity. Therefore, more than one gene may be involved in the resistance and susceptibility to EAE, since final mortality rates for actively immunized F 2 hybrids were much less than that for F 1's. (Dr. Stone and Ms. Amsbaugh.)

Comparison of a Human BCGF with Murine BCGF-II

TH2.2 is a lymphocyte hybridoma which is induced to differentiate and secrete IgM by exposure to LPS. The differentiation was shown to be terminal in a limiting dilution cloning assay, with the rate of secretion of IgM inversely proportional to the number of cells in the terminal clones. Conditioned medium from uninduced TH 2.2 cells reduces the number terminally differentiated clones by several fold, and reduces the rate of secretion of IgM in the remaining differentiated cells. In addition TH 2.2 induced with LPS produced lymphokine (s) with biological activity resembling GM CSF. The GM CSF, like the IgM was produced mainly by terminally differentiated clones. No GM CSF activity was found in the smallest clones (@100 cells). In larger terminal clones [GM CSF] per 1000 cells was very strongly correlated with [IgM] per 1000 cells. These results show that (1) autoregulatory activities of B cells described by others also strongly influence (inhibit) differentiation of B cells; (2) that some B cells may be capable of producing a number of different lymphokines, and that stimulation (induction) can change this production; and (3) that LPS can induce 2 differentiative events in individual clones of B cells. (Drs. Sulis, Asofsky, Drs. Pluznick, Dicke NIDR). (Drs. Ennist & Howard.)

Differentiation of Murine B Cell hybridomas

A human BCGF, induced proliferation in resting murine small B lymphocytes, as well as augmenting the response of such lymphocytes activated with dextran sulfate. In addition, the human BCGF augmented the response of murine cells to anti-Ig and BSF-1, although it was inactive in the absence of BSF-1. Human BCGF, not murine BCGF-II had this activity. Murine BCGF-II but not human BCGF stimulated BCL-1 lymphoma cells to division, suggesting the existence of two BCGF-II activities. (Drs. Sulis, Asofsky, Drs. Pluznick, Dicke NIDR.) (Drs. Ennist and Howard.)

Administrative

Five postdoctoral scientists completed their fellowships: Dr. M. Howard, Visiting Scientist; Dr. T. Teranishi, Visiting Associate; Dr. G. Wesley, Medical Staff Fellow, Dr. P. Dubois, NATO Investigator; and Dr. C. Sulis, Guest Researcher. Dr. J., Hiernaux, a Visiting Associate arrived during the summer. Dr. T. M. Chused and his staff were transferred administratively to LI, but retained their laboratory space in LMI.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00131-19 LMI
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>Mechanism of hypersensitivity in inbred histocompatible guinea pigs</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: S.H. Stone Others: D.F. Amsbaugh M.B. Datiles* C.S. Raine**	Head, Experimental Autoimmunity Section Biologist	LMI, NIAID LMI, NIAID
COOPERATING UNITS (if any) *Clinical Branch and Laboratory of Vision Research, NEI; **Division of Neuro-pathology, Albert Einstein College of Medicine, New York, NY		
LAB/BRANCH Laboratory of Microbial Immunity		
SECTION Experimental Autoimmunity Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 2.0	PROFESSIONAL: 1.0	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.) We have been studying genetic differences in susceptibility to autoimmune encephalomyelitis (EAE) between Strain 13 (susceptible) and Strain 2 (resistant) guinea pigs. Strain 2 sensitized to guinea pig spinal cord antigen shows histological evidence but not clinical signs of EAE. Both genetic and immunological approaches to finding the basis for this difference have been undertaken. Final mortality rates for F ₂ hybrids were much less than for fully susceptible F ₁ 's bringing up the question whether multiple genes are involved in determination of susceptibility to EAE. Adoptive transfers of sensitized T cells to F ₁ recipients showed that 13 but not 2 donor cells regularly induce clinical disease. Strain 2 lymphocytes can easily transfer tuberculin hypersensitivity and cause histological changes, but not clinical signs without using enormous numbers of donor T cells purified by panning. This indicates a cytotoxic factor capable of damaging neural capacity is in short supply in Strain 2 T cells.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00134-24 LMI
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>Control of Immunoglobulin Synthesis in Mice</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: Richard Asofsky Others: Carol Sulis Teranishi Tsuyoski David Greenblatt Ada Brooks Maureen Howard Dov Pluznick Mathias Dickel	Chief, Guest Researcher Visiting Associate Guest Researcher Biological Lab. Tech. Visiting Scientist Visiting Scientist Visiting Scientist	LMI, NIAID LMI, NIAID LMI, NIAID LMI, NIAID LMI, NIAID LMI, NIAID LMI, NIAID NIDR
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Microbial Immunity		
SECTION		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 3.0	PROFESSIONAL: 2.0	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>TH2.2 a mouse B cell hybridoma is induced to terminal differentiation by LPS. The terminally differentiated clones secrete IgM in inverse proportion to the number of cells in the clone. Terminal differentiation and secretion of immunoglobulin were partly reversed by incubation of induced cells in conditioned medium from uninduced cells.</p> <p>The terminally differentiated cells also produced a lymphokine with activity of GM-CSF. In terminal clones of intermediate size there was a strong correlation between [IgM]/1000 cells and [CSF]/1000 cells; e.g. The LPS was inducing 2 different activities in the same clones.</p> <p>F.16.47 is a B cell hybrid which responds to small doses of BSF-1 with a rapid (12 hours) and large (10 fold) increase in mRNA for Ia polypeptides, followed by an increase in expression in Ia antigens on the membrane. Molecularly cloned BSF-1 produced this effect.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00136-14 LMI
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Characterization and Differentiation of Thymic Lymphocytes		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: B.J. Fowlkes, Microbiologist, LMI, NIAID Others: Linette Edison, B.S., OSD, NIAID Ronald Germain, M.D., LI, NIAID Thomas Chused, M.D., PhD, LI, NIAID Ronald Schwartz, M.D., LI, NIAID Ada Kruisbeek, PhD, DCT, NCI Andrew Lew, PhD, LIC, NIAID Lawrence Samelson, M.D., DBMD, NICHD Jeff Bluestone, PhD, IB, NCI		
COOPERATING UNITS (if any) David Raulet, PhD, Massachusetts Institute of Technology Cambridge, MA Bonnie Mathieson, PhD, FCRF, NCI		
LAB/BRANCH Laboratory of Microbial Immunity		
SECTION Experimental Pathology		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> Several studies are in progress aimed at determining the growth, differentiation, and cellular interactions involved in T cell development and in the inductive signals and selective mechanism which shape the mature T cell antigen repertoire. Mice treated <u>in utero</u> with anti-IL-2 receptor antibodies do not produce mature T cells in the fetal thymus and fail to express any TcRα mRNA in cortical-type thymocytes. Also the majority of proliferating cells in the early fetal thymus express IL-2 and IL-2R mRNA. The importance of the IL-2 and IL-2R to T cell development was also demonstrated in studies using PMA and ionomycin which promotes growth, IL-2 secretion, and differentiation to a mature phenotype with TcRα mRNA of the early adult and fetal thymocytes. Antibodies to the IL-2R block this growth and differentiation. No mature T cell antigen receptor or T3 was expressed in cells differentiated in response to PMA and ionophore. However, when the same cells are stimulated to grow <u>in vitro</u> using a combination of IL-1, IL-2, IL-3, and other factors, 100% T3⁺ cells develop by either selection or differentiation, a proportion of which express a mature phenotype (Lyt2⁺, L3T4⁻). The cells do not express the α, β heterodimeric TcR on their surface but instead express a δ, δ structure. This is the first demonstration of chain expression at the protein level in the mouse. The δ chain expressed on these cells has yet to be characterized. A cell with the same phenotype and TcR structure is first obtained in a minor subpopulation of day 15 fetal and in immature adult thymocytes. A study using <u>in situ</u> hybridization has revealed the ontogenetic expression of six important T cell genes at much earlier time points than was previously possible. More data have been obtained on rearrangements, nonconventional rearrangements, and of differential V region useage in fetal and immature adult thymocytes or in hybrids resulting from using these cells. Also studies are in progress of the T3 components and on the phosphorylation of these components as a result of activation. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00141-10 LMI
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Immune Responses to Malaria		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) P.I. Richard Asofsky		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Microbial Immunity		
SECTION		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20792		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Inactive in the Present year.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00143-17 LMI
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Genetic control of the antibody response to microbial antigens		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: F.J. Baker, Head, Microbiologist and Immunology Section, LMI, NIAID		
Others: K. Elkins, Staff Fellow LMI, NIAID T.M. Chused, Senior Investigator, LMI, NIAID P.W. Stashak, Microbiologist, LMI, NIAID M. Fauntleroy, Biologist, LMI, NIAID		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Microbial Immunity		
SECTION Microbiology and Immunology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 1.3	PROFESSIONAL: 0.6	OTHER: 0.7
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <u>Immune B cells</u> from various strains of inbred mice activate <u>suppressor T cells</u> after transfer to recipients of the same strain. However, this does not occur using immune B cells obtained from either young or old NZB/N mice, even though suppressor T cells can be activated <u>in situ</u> upon priming with a low dose of antigen. This suggests major differences between NZB/N and other strains of mice in either the homing patterns for immune B cells or the manner in which suppressor T cells are activated. The expression of <u>amplifier and helper T cell activity</u> was examined in NZB/N mice of different ages. Loss of suppressor T cell activity and increased amplifier T cell activity coincided with the development of <u>autoimmune disease</u> in aging NZE/N mice. Helper T cell activity was present in young NZB/N mice; however, it was absent in old NZB/N mice expressing maximal amplifier T cell activity. This provides additional support for the fact that amplifier and helper functions are mediated by different sub-populations of T cells.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00144-22 LMI
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Regulation of the antibody response to microbial polysaccharide antigens		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	P.J. Baker,	Head, Microbiology and Immunology Section, IMI, NIAID
Others:	K. Elkins,	Staff Fellow, IMI, NIAID
	Jon A. Rudbach*	
	P.W. Stashak,	Microbiologist, IMI, NIAID
	M. Fauntleroy,	Biologist, IMI, NIAID
COOPERATING UNITS (if any) *Ribi ImmunoChem Inc., Hamilton, MT.		
LAB/BRANCH Laboratory of Microbial Immunity		
SECTION Microbiology and Immunology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD. 20892		
TOTAL MAN-YEARS: 1.3	PROFESSIONAL: 0.7	OTHER: 0.6
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <u>Regulation of IgM antibody responses to several bacterial lipopolysaccharides, including Escherichia coli 0113 LPS (EC 0113-LPS), E.coli 055 LPS (EC 055-LPS) and Serratia marcesans LPS (SM-LPS) was examined. Memory to EC 0113-LPS was generated after exposure to a single subimmunogenic dose of this antigen; it developed in a cyclic manner and persisted for at least 20 days. By contrast, mice primed with a subimmunogenic dose of EC 055 LPS or SM-LPS developed profound suppression rather than memory for the antibody response to the same LPS used for priming; the development of such suppression was dose and time dependent and not due to a switch in immunoglobulin isotype. The cellular mechanisms involved in the expression of memory and suppression to LPS are being investigated in detail. The results of other studies showed that polyclonal stimulation by LPS results in the preferential activation of memory, rather than virgin B cells.</u>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00145-19 LMI
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Mode of action of thymus-derived (T) suppressor and amplifier cells		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: P.J. Baker, Head, Microbiology & Immunology Section, LMI, NIAID Others: K. Elkins, Staff Fellow LMI, NIAID P.W. Stashak, Microbiologist, LMI, NIAID B. Prescott*		
COOPERATING UNITS (if any) *Biomedical Research Institute, Rockville, MD. 20852		
LAB/BRANCH Laboratory of Microbial Immunity		
SECTION Microbiology and Immunology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 1.4	PROFESSIONAL: 0.7	OTHER: 0.7
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <u>Regulatory T cells</u> involved in the <u>antibody response</u> to <u>Type III pneumococcal polysaccharide (SSS-III)</u> can be activated by the infusion of <u>B cells</u> primed by prior exposure to antigen (SSS-III). If such immune B cells are incubated before transfer-with appropriate amounts of <u>F(ab')₂</u> fragments from anti-IgM and IgG (heavy and light chain specific) <u>antibody</u> , or anti-IgM (μ chain specific) <u>antibody</u> , the transferred B cells fail to act as inducers for the activation of <u>suppressor T cells</u> . By contrast, the treatment of immune B cells with <u>F(ab')₂</u> of <u>anti-IgD</u> (delta chain specific) is without effect. Since both antibody treated and untreated B cells localized to the spleen in the same manner after cell transfer, these findings indicate that B cell surface antibody of the IgM class plays an important role in the activation of suppressor T cells involved in the antibody response to SSS-III.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00146-12 LMI
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>Immunological Studies of Components Isolated from Bacteria, Parasites, and Plants</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: G. Caldes, Chemist LMI, NIAID Others: P.J. Baker, Head, Microbiology and Immunology Section, LMI, NIAID B. Prescott* P.W. Stashak, Microbiologist LMI, NIAID M. Fauntleroy, Biologist, LMI, NIAID		
COOPERATING UNITS (if any) *Liomedical Research Institute, Rockville, ND. 20852		
LAB/BRANCH Laboratory of Microbial Immunity		
SECTION Microbiology and Immunology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) This project was discontinued as of October 30th, 1985 with the retirement of the Principal Investigator, Mr. G. Caldes.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00153-09 LMI
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>In Vitro Responses of Human Peripheral Leukocytes</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) P.I. Chu Hsia ^o Visiting Fellow LMI, NIAID Other: Richard Asofsky Chief LMI, NIAID		
COOPERATING UNITS (if any)		
LAB/BRANCH <u>Laboratory of Microbial Immunity</u> SECTION		
INSTITUTE AND LOCATION <u>NIAID, NIH, Bethesda, MD 20892</u>		
TOTAL MAN-YEARS: 1.1	PROFESSIONAL: 1.1	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) We have obtained a human x mouse hybridoma, C3A32 produced by using human lymphocytes immunized <u>in vitro</u> with tetanus toxoid, then fused with mouse myeloma line. The hybridoma secretes human IgM antitetanus antibody. Cells of clone C3A2 have the following characteristics: (1) they stain with both labeled anti-IgG and anti-IgM. The anti-IgG is blocked by Hu IgG only; the anti-IgM is blocked by IgM only. In ELISA assays most clones of C3A2 produce anti-tetanus anti-body with antigenic characteristics of both IgG and IgM. The immunoglobulin produced appears to be a single species of macroglobulin (IgM) with some antigenic characteristics of IgG not founded in human serum IgM.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00423-03 LMI
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Factor Mediated Regulation of B cell Growth and Differentiation		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Maureen Howard Visiting Scientist LMI, NIAID Others: David Ennist Staff Fellow LMI, NIAID George Wesley Medical Staff Fellow LMI, NIAID David Greenblatt Medical Staff Fellow LMI, NIAID Philippe Dubois Guest Researcher LMI, NIAID Peter Stein Guest Reseracher LMI, NIAID Abby Maize* Surendra Shama* Timothy Mossman** Robert Coffman**		
COOPERATING UNITS (if any) *M.D. Anderson Hospital, Houston, TX **DNAX Research Institute		
LAB/BRANCH Laboratory of Microbial Immunity		
SECTION		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS 2.9	PROFESSIONAL: 2.9	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The proliferative response of antigen specific (for TNP) B lymphocytes to TI-2 antigens (eg TNP-Ficoll) shows an absolute requirement for BSF-1, and is enhanced by IL-1. Such responses to T dependent antigens (e.g. TNP-OVA) requires direct interaction with carrier specific T cells as well as BSF-1. Such responses are suppressed by IFN.</p> <p>2. A human BCGF. induced proliferation in resting murine small B lymphocytes, as well as augmenting the response of such lymphocytes activated with dextran sulfate. In addition, the human BCGF augmented the response of murine cells to anti-Ig and BSF-1, although it was inactive in the absence of BSF-1. Human BCGF, not murine BCGF-II had this activity. Murine BCGF-II but not human BCGF stimulated BCL-1 lymphoma cells to division, suggesting the existence of two BCGF-II activities.</p>		

10 1
11 2
12 3
13 4
14 5
15 6
16 7
17 8
18 9
19 10
20 11
21 12
22 13
23 14
24 15
25 16
26 17
27 18
28 19
29 20
30 21
31 22
32 23
33 24
34 25
35 26
36 27
37 28
38 29
39 30
40 31
41 32
42 33
43 34
44 35
45 36
46 37
47 38
48 39
49 40
50 41
51 42
52 43
53 44
54 45
55 46
56 47
57 48
58 49
59 50
60 51
61 52
62 53
63 54
64 55
65 56
66 57
67 58
68 59
69 60
70 61
71 62
72 63
73 64
74 65
75 66
76 67
77 68
78 69
79 70
80 71
81 72
82 73
83 74
84 75
85 76
86 77
87 78
88 79
89 80
90 81
91 82
92 83
93 84
94 85
95 86
96 87
97 88
98 89
99 90
100 91
101 92
102 93
103 94
104 95
105 96
106 97
107 98
108 99
109 100
110 101
111 102
112 103
113 104
114 105
115 106
116 107
117 108
118 109
119 110
120 111
121 112
122 113
123 114
124 115
125 116
126 117
127 118
128 119
129 120
130 121
131 122
132 123
133 124
134 125
135 126
136 127
137 128
138 129
139 130
140 131
141 132
142 133
143 134
144 135
145 136
146 137
147 138
148 139
149 140
150 141
151 142
152 143
153 144
154 145
155 146
156 147
157 148
158 149
159 150
160 151
161 152
162 153
163 154
164 155
165 156
166 157
167 158
168 159
169 160
170 161
171 162
172 163
173 164
174 165
175 166
176 167
177 168
178 169
179 170
180 171
181 172
182 173
183 174
184 175
185 176
186 177
187 178
188 179
189 180
190 181
191 182
192 183
193 184
194 185
195 186
196 187
197 188
198 189
199 190
200 191
201 192
202 193
203 194
204 195
205 196
206 197
207 198
208 199
209 200
210 201
211 202
212 203
213 204
214 205
215 206
216 207
217 208
218 209
219 210
220 211
221 212
222 213
223 214
224 215
225 216
226 217
227 218
228 219
229 220
230 221
231 222
232 223
233 224
234 225
235 226
236 227
237 228
238 229
239 230
240 231
241 232
242 233
243 234
244 235
245 236
246 237
247 238
248 239
249 240
250 241
251 242
252 243
253 244
254 245
255 246
256 247
257 248
258 249
259 250
260 251
261 252
262 253
263 254
264 255
265 256
266 257
267 258
268 259
269 260
270 261
271 262
272 263
273 264
274 265
275 266
276 267
277 268
278 269
279 270
280 271
281 272
282 273
283 274
284 275
285 276
286 277
287 278
288 279
289 280
290 281
291 282
292 283
293 284
294 285
295 286
296 287
297 288
298 289
299 290
300 291
301 292
302 293
303 294
304 295
305 296
306 297
307 298
308 299
309 300
310 301
311 302
312 303
313 304
314 305
315 306
316 307
317 308
318 309
319 310
320 311
321 312
322 313
323 314
324 315
325 316
326 317
327 318
328 319
329 320
330 321
331 322
332 323
333 324
334 325
335 326
336 327
337 328
338 329
339 330
340 331
341 332
342 333
343 334
344 335
345 336
346 337
347 338
348 339
349 340
350 341
351 342
352 343
353 344
354 345
355 346
356 347
357 348
358 349
359 350
360 351
361 352
362 353
363 354
364 355
365 356
366 357
367 358
368 359
369 360
370 361
371 362
372 363
373 364
374 365
375 366
376 367
377 368
378 369
379 370
380 371
381 372
382 373
383 374
384 375
385 376
386 377
387 378
388 379
389 380
390 381
391 382
392 383
393 384
394 385
395 386
396 387
397 388
398 389
399 390
400 391
401 392
402 393
403 394
404 395
405 396
406 397
407 398
408 399
409 400
410 401
411 402
412 403
413 404
414 405
415 406
416 407
417 408
418 409
419 410
420 411
421 412
422 413
423 414
424 415
425 416
426 417
427 418
428 419
429 420
430 421
431 422
432 423
433 424
434 425
435 426
436 427
437 428
438 429
439 430
440 431
441 432
442 433
443 434
444 435
445 436
446 437
447 438
448 439
449 440
450 441
451 442
452 443
453 444
454 445
455 446
456 447
457 448
458 449
459 450
460 451
461 452
462 453
463 454
464 455
465 456
466 457
467 458
468 459
469 460
470 461
471 462
472 463
473 464
474 465
475 466
476 467
477 468
478 469
479 470
480 471
481 472
482 473
483 474
484 475
485 476
486 477
487 478
488 479
489 480
490 481
491 482
492 483
493 484
494 485
495 486
496 487
497 488
498 489
499 490
500 491
501 492
502 493
503 494
504 495
505 496
506 497
507 498
508 499
509 500
510 501
511 502
512 503
513 504
514 505
515 506
516 507
517 508
518 509
519 510
520 511
521 512
522 513
523 514
524 515
525 516
526 517
527 518
528 519
529 520
530 521
531 522
532 523
533 524
534 525
535 526
536 527
537 528
538 529
539 530
540 531
541 532
542 533
543 534
544 535
545 536
546 537
547 538
548 539
549 540
550 541
551 542
552 543
553 544
554 545
555 546
556 547
557 548
558 549
559 550
560 551
561 552
562 553
563 554
564 555
565 556
566 557
567 558
568 559
569 560
570 561
571 562
572 563
573 564
574 565
575 566
576 567
577 568
578 569
579 570
580 571
581 572
582 573
583 574
584 575
585 576
586 577
587 578
588 579
589 580
590 581
591 582
592 583
593 584
594 585
595 586
596 587
597 588
598 589
599 590
600 591
601 592
602 593
603 594
604 595
605 596
606 597
607 598
608 599
609 600
610 601
611 602
612 603
613 604
614 605
615 606
616 607
617 608
618 609
619 610
620 611
621 612
622 613
623 614
624 615
625 616
626 617
627 618
628 619
629 620
630 621
631 622
632 623
633 624
634 625
635 626
636 627
637 628
638 629
639 630
640 631
641 632
642 633
643 634
644 635
645 636
646 637
647 638
648 639
649 640
650 641
651 642
652 643
653 644
654 645
655 646
656 647
657 648
658 649
659 650
660 651
661 652
662 653
663 654
664 655
665 656
666 657
667 658
668 659
669 660
670 661
671 662
672 663
673 664
674 665
675 666
676 667
677 668
678 669
679 670
680 671
681 672
682 673
683 674
684 675
685 676
686 677
687 678
688 679
689 680
690 681
691 682
692 683
693 684
694 685
695 686
696 687
697 688
698 689
699 690
700 691
701 692
702 693
703 694
704 695
705 696
706 697
707 698
708 699
709 700
710 701
711 702
712 703
713 704
714 705
715 706
716 707
717 708
718 709
719 710
720 711
721 712
722 713
723 714
724 715
725 716
726 717
727 718
728 719
729 720
730 721
731 722
732 723
733 724
734 725
735 726
736 727
737 728
738 729
739 730
740 731
741 732
742 733
743 734
744 735
745 736
746 737
747 738
748 739
749 740
750 741
751 742
752 743
753 744
754 745
755 746
756 747
757 748
758 749
759 750
760 751
761 752
762 753
763 754
764 755
765 756
766 757
767 758
768 759
769 760
770 761
771 762
772 763
773 764
774 765
775 766
776 767
777 768
778 769
779 770
780 771
781 772
782 773
783 774
784 775
785 776
786 777
787 778
788 779
789 780
790 781
791 782
792 783
793 784
794 785
795 786
796 787
797 788
798 789
799 790
800 791
801 792
802 793
803 794
804 795
805 796
806 797
807 798
808 799
809 800
810 801
811 802
812 803
813 804
814 805
815 806
816 807
817 808
818 809
819 810
820 811
821 812
822 813
823 814
824 815
825 816
826 817
827 818
828 819
829 820
830 821
831 822
832 823
833 824
834 825
835 826
836 827
837 828
838 829
839 830
840 831
841 832
842 833
843 834
844 835
845 836
846 837
847 838
848 839
849 840
850 841
851 842
852 843
853 844
854 845
855 846
856 847
857 848
858 849
859 850
860 851
861 852
862 853
863 854
864 855
865 856
866 857
867 858
868 859
869 860
870 861
871 862
872 863
873 864
874 865
875 866
876 867
877 868
878 869
879 870
880 871
881 872
882 873
883 874
884 875
885 876
886 877
887 878
888 879
889 880
890 881
891 882
892 883
893 884
894 885
895 886
896 887
897 888
898 889
899 890
900 891
901 892
902 893
903 894
904 895
905 896
906 897
907 898
908 899
909 900
910 901
911 902
912 903
913 904
914 905
915 906
916 907
917 908
918 909
919 910
920 911
921 912
922 913
923 914
924 915
925 916
926 917
927 918
928 919
929 920
930 921
931 922
932 923
933 924
934 925
935 926
936 927
937 928
938 929
939 930
940 931
941 932
942 933
943 934
944 935
945 936
946 937
947 938
948 939
949 940
950 941
951 942
952 943
953 944
954 945
955 946
956 947
957 948
958 949
959 950
960 951
961 952
962 953
963 954
964 955
965 956
966 957
967 958
968 959
969 960
970 961
971 962
972 963
973 964
974 965
975 966
976 967
977 968
978 969
979 970
980 971
981 972
982 973
983 974
984 975
985 976
986 977
987 978
988 979
989 980
990 981
991 982
992 983
993 984
994 985
995 986
996 987
997 988
998 989
999 990
1000 991
1001 992
1002 993
1003 994
1004 995
1005 996
1006 997
1007 998
1008 999
1009 1000
1010 1001
1011 1002
1012 1003
1013 1004
1014 1005
1015 1006
1016 1007
1017 1008
1018 1009
1019 1010
1020 1011
1021 1012
1022 1013
1023 1014
1024 1015
1025 1016
1026 1017
1027 1018
1028 1019
1029 1020
1030 1021
1031 1022
1032 1023
1033 1024
1034 1025
1035 1026
1036 1027
1037 1028
1038 1029
1039 1030
1040 1031
1041 1032
1042 1033
1043 1034
1044 1035
1045 1036
1046 1037
1047 1038
1048 1039
1049 1040
1050 1041
1051 1042
1052 1043
1053 1044
1054 1045
1055 1046
1056 1047
1057 1048
1058 1049
1059 1050
1060 1051
1061 1052
1062 1053
1063 1054
1064 1055
1065 1056
1066 1057
1067 1058
1068 1059
1069 1060
1070 1061
1071 1062
1072 1063
1073 1064
1074 1065
1075 1066
1076 1067
1077 1068
1078 1069
1079 1070
1080 1071
1081 1072
1082 1073
1083 1074
1084 1075
1085 1076
1086 1077
1087 1078
1088 1079
1089 1080
1090 1081
1091 1082
1092 1083
1093 1084
1094 1085
1095 1086
1096 1087
1097 1088
1098 1089
1099 1090
1100 1091
1101 1092
1102 1093
1103 1094
1104 1095
1105 1096
1106 1097
1107 1098
1108 1099
1109 1100
1110 1101
1111 1102
1112 1103
1113 1104
1114 1105
1115 1106
1116 1107
1117 1108
1118 1109
1119 1110
1120 1111
1121 1112
1122 1113
1123 1114
1124 1115
1125 1116
1126 1117
1127 1118
1128 1119
1129 1120
1130 1121
1131 1122
1132 1123
1133 1124
1134 1125
1135 1126
1136 1127
1137 1128
1138 1129
1139 1130
1140 1131
1141 1132
1142 1133
1143 1134
1144 1135
1145 1136
1146 1137
1147 1138
1148 1139
1149 1140
1150 1141
1151 1142
1152 1143
1153 1144
1154 1145
1155 1146
1156 1147
1157 1148
1158 1149
1159 1150
1160 1151
1161 1152
1162 1153
1163 1154
1164 1155
1165 1156
1166 1157
1167 1158
1168 1159
1169 1160
1170 1161
1171 1162
1172 1163
1173 1164
1174 1165
1175 1166
1176 1167
1177 1168
1178 1169
1179 1170
1180 1171
1181 1172
1182 1173
1183 1174
1184 1175
1185 1176
1186 1177
1187 1178
1188 1179
1189 1180
1190 1181
1191 1182
1192 1183
1193 1184
1194 1185
1195 1186
1196 1187
1197 1188
1198 1189
1199 1190
1200 1191
1201 1192
1202 1193
1203 1194
1204 1195
1205 1196
1206 1197
1207 1198
1208 1199
1209 1200
1210 1201
1211 1202
1212 1203
1213 1204
1214 1205
1215 1206
1216 1207
1217 1208
1218 1209
1219 1210
1220 1211
1221 1212
1222 1213
1223 1214
1224 1215
1225 1216
1226 1217
1227 1218
1228 1219
1229 1220
1230 1221
1231 1222
1232 1223
1233 1224
1234 1225
1235 1226
1236 1227
1237 1228
1238 1229
1239 1230
1240 1231
1241 1232
1242 1233
1243 1234
1244 1235
1245 1236
1246 1237
1247 1238
1248 1239
1249 1240
1250 1241
1251 1242
1252 1243
1253 1244
1254 1245
1255 1246
1256 1247
1257 1248
1258 1249
1259 1250
1260 1251
1261 1252
1262 1253
1263 1254
1264 1255
1265 1256
1266 1257
1267 1258
1268 1259
1269 1260
1270 1261
1271 1262
1272 1263
1273 1264
1274 1265
1275 1266
1276 1267
1277 1268
1278 1269
1279 1270
1280 1271
1281 1272
1282 1273
1283 1274
1284 1275
1285 1276
1286 1277
1287 1278
1288 1279
1289 1280
1290 1281
1291 1282
1292 1283
1293 1284
1294 1285
1295 1286
1296 1287
1297 1288
1298 1289
1299 1290
1300 1291
1301 1292
1302 1293
1303 1294
1304 1295
1305 1296
1306 1297
1307 1298
1308 1299
1309 1300
1310 1301
1311 1302
1312 1303
1313 1304
1314 1305
1315 1306
1316 1307
1317 1308
1318 1309
1319 1310
1320 1311
1321 1312
1322 1313
1323 1314
1324 1315
1325 1316
1326 1317
1327 1318
1328 1319
1329 1320
1330 1321
1331 1322
1332

LABORATORY OF MOLECULAR MICROBIOLOGY
1986 Annual Reports
Table of Contents

<u>Z01 AI</u> <u>Project Number</u>	<u>Title</u>	<u>Page</u>
Summary		10-1
00011-20	Studies of Small DNA Containing Viruses Belonging to the Family Parvoviridae - Hoggan (Terminated)	10-13
00027-19	Basic Studies of Mycoplasmas - Tully	10-14
00190-08	The Molecular Genetics of Eukaryotic Cells and Their Viruses - Martin	10-15
00218-05	Biochemical and Chemical Studies on Retroviral DNA - Repaske (Revised Title)	10-16
00219-05	Molecular and Genetic Analysis of Streptococci - LeBlanc	10-17
00222-05	Characterization of Endogenous Ecotropic and Xenotropic Murine Leukemia Virus - Theodore	10-18
00281-05	Molecular Cloning of Recombinant MuLV Proviral Sequences in Inbred Mice - Buckler	10-19
00300-05	Control of Resistance to Viral Leukemogenesis in Wild Mouse Population - Kozak	10-20
00301-05	Cellular Genes Involved in Transformation and MuLV Transmission in Inbred Mice - Kozak	10-21
00304-05	Pathogenesis of Disease Induced by Friend MuLV - Silver	10-22
00353-04	Structural and Functional Analysis of Endogenous Proviruses of Mice - Khan (Revised Title)	10-23
00388-03	Functional Analysis of Human Retroviral Genes - Rabson	10-24
00395-03	Genetic and Molecular Analysis of Anaerobic Bacteria Indigenous to Humans - Smith	10-25
00415-03	The Molecular Biology of Retroviruses Associated with AIDS - Martin	10-26
00433-02	Use of Retroviruses as Tagged Insertional Mutagens - Silver	10-27

<u>Project Number</u>	<u>Title</u>	<u>Page</u>
00434-02	Studies on the Dissemination of Streptococcal Antibiotic Resistance Determinants - LeBlanc	10-28
00437-02	The Biology and Genetics of the AIDS Retrovirus - Hoggan	10-29
00438-02	The Molecular Biology of Cellular Oncogenes - Santos	10-30
00464-01	Plasmid Replication and Expression in Streptococci - Ranhand (New)	10-31
00466-01	Transcriptional Complexity of AIDS Retrovirus - Venkatesan (New)	10-32
00467-01	Structure and Function of Polypeptides Encoded by AIDS RV Genome - Venkatesan (New)	10-33

Public Health Service - National Institutes of Health
SUMMARY REPORT

ANNUAL REPORT OF THE LABORATORY OF MOLECULAR MICROBIOLOGY, NIAID
October 1, 1985 - September 30, 1986

Dr. Malcolm A. Martin, Chief

The Laboratory of Molecular Microbiology (LMM), established in May, 1981, applies molecular biological techniques to study the structure and regulation of prokaryotic and eukaryotic genes. Programmatically, a wide range (retroviruses, fungi, streptococci, anaerobic bacteria, and mycoplasmas) of microorganisms and their host cells are investigated. In addition, the administration of this diverse group presents a logistic challenge since LMM laboratories are located in two different buildings in Bethesda and one building in Frederick. The Bacterial Virulence and Mycoplasma Sections (headed by Drs. LeBlanc and Tully, respectively) as well as their professional and support staff occupy Building 550, FCRF. Members of the Biochemical Virology and Viral Biology Sections have space in Buildings 5 and 7 on the Bethesda campus. Although the research activities conducted by LMM staff are quite diverse, a common theme permeating most of the programs centers on the characterization of genetic elements (both chromosomal and episomal) involved in the interaction of microorganisms and their host cells.

For the past two years, a major research effort of the LMM scientific staff has focused on AIDS. This work continues to be carried out as a collaborative effort with Drs. Tom Folks, Clifford Lane, and Anthony Fauci of the Laboratory of Immunoregulation, who provide expertise in cell biology and clinical immunology. An early product of this concerted effort has been the development of a continuous growing, IL-2 independent human T lymphocyte line (A3.01) for the efficient propagation of the AIDS retrovirus (RV). Molecular clones of the AIDS proviral DNA were isolated from a human lambda phage gene library constructed from infected A3.01 cells and used: 1) to identify and map the multiple species of viral mRNAs; 2) to demonstrate that no two AIDS RV isolates were the same; and 3) to develop sensitive *in situ* hybridization techniques for the detection of the rare (1 in 10^5) cell in clinical specimens synthesizing AIDS RV nucleic acid. During the past year, sensitive methods have been developed for the titration of virus preparations and to monitor the biological activity of viral DNA following its introduction into mammalian cells. Transfection experiments demonstrated that AIDS virus proteins could be synthesized and assembled into progeny particles in a variety of non-lymphocyte vertebrate cells including those of mouse, monkey and man. *In vitro* mutagenesis procedures were used to generate mutations affecting the envelope, endonuclease, "A", and *tat* genes of the virus. cDNA clones representing the messenger RNAs encoded by the "A", "B", and *tat* open reading frames were obtained and analyzed functionally. Cell lines have been established that constitutively express specific viral proteins to establish their function(s) during cytocidal and latent infections. Finally, systems have been developed to evaluate the interaction of other animal viruses with the AIDS RV.

LMM research endeavors involving retroviruses date back to 1979 when collaborative studies with the late Dr. Wallace P. Rowe commenced. Activities in the area of murine retroviruses continue to flourish. Cloned DNA segments that clearly discriminate the closely related xenotropic and mink cell focus-inducing (MCF) murine leukemia virus (MuLV) envelope genes were recently obtained and used to demonstrate that a majority of the endogenous MuLV segments present in the genomes of inbred strains of laboratory mice contain MCF-related (rather than xenotropic) env sequences. In contrast, most strains of wild mice contain few, if any, copies of endogenous retroviral sequences related to murine leukemia viruses. Since these animals harbor inducible proviruses with novel, biological and biochemical properties, they represent a valuable resource for evaluating the evolution and genetics of mammalian retroviruses.

In a series of experiments designed to assess the extent to which streptococcal antibiotic resistance genes have been disseminated in the natural environment, members of the Bacterial Virulence Section have shown that genetic determinants encoding resistance to streptomycin, kanamycin and erythromycin, originally found on the extensively studied S. faecalis plasmid pJH1, were also present on novel plasmids in nearly 70% of multiple drug resistant human and animal isolates of group D streptococci. A novel spectinomycin resistance gene was cloned from plasmid DNA carried by a human clinical isolate of S. faecalis. Additional studies indicated that this determinant was present in S. faecalis isolates of animal origin prior to its emergence in the human strain. Considerable progress has also been made in studies of antibiotic resistance plasmids in the anaerobic bacterium, Bacteroides fragilis. Transposon-like structures encoding clindamycin resistance on three different Bacteroides R plasmids were cloned in E. coli. All three structures were shown to be bounded by a homologous 1.2 kb directly repeated sequence (DRS).

Specific Research Accomplishments

Development of DNA probes for MCF, xenotropic and amphotropic MuLV env genes. DNA sequences (100 bp) from analogous portions of the MCF, xenotropic and amphotropic gp70 env coding regions were isolated and subcloned into an M13 phage vector. No cross reactivity could be demonstrated in Southern blot hybridization of cloned proviral DNAs. An analysis of DNAs prepared from inbred and wild mice indicated that DNAs of inbred mice contain 20-30 MCF env copies. Many wild mouse species contained neither env gene, M. m. domesticus contained MCF env genes, and M. m. Musculus, M. molossinus and M. custanus contained copies of xenotropic env segments. No mice were found to contain endogenous copies of amphotropic env related genes including mice from Lake Casitas, CA, which harbor infectious amphotropic virus. (O'Neill, Repaske, Kozak).

Restriction mapping of Bxv-1. Bxv-1 is the major inducible xenotropic proviral locus of common laboratory mice. Through the use of somatic cell hybrids in conjunction with the xenotropic env specific probe, a restriction map was derived of the internal structure and flanking cell sequences of Bxv-1. Several lines of evidence indicate that the enhancer region within the LTRs of leukemogenic MCF viruses of AKR mice is probably acquired from the Bxv-1 locus. (Kozak, O'Neill, Repaske).

Characterization of wild mice for sensitivity to MuLVs. Many wild mice lack the Fv-1-type restriction of MuLVs characteristic of inbred mice. These wild mice carry a novel non-restrictive allele at Fv-1. Additional experiments have demonstrated that wild mouse populations from Maryland and Delaware also differ from laboratory mice since cells from these mice restrict NB-tropic viruses. It is not yet known whether this novel phenotype is due to an allelic variant at the Fv-1 locus. (Kozak).

The LTR and 3' pol regions of leukemogenic and non-leukemogenic MCF MuLVs contain significant nucleotide differences. Infectious molecular clones of leukemogenic MCF-13 and non-leukemogenic MCF-111A proviral DNAs were obtained and the nucleotide sequence of the LTRs, 3' pol and env regions was determined. The results of comparative sequence analysis indicated: the LTR associated with MCF-13 is similar to that present in xenotropic MuLVs, whereas the LTR sequence of MCF-111A is virtually identical (1 bp difference) to the LTR associated with ecotropic MuLVs; no significant sequence divergence was seen in the env regions of MCF-13 and MCF-111A; a 12 bp nucleotide stretch, characteristic of leukemogenic MCF MuLVs, was conserved in the 3' pol region of MCF-13 but was lacking in MCF-111A. These results suggest that the leukemogenic potential of leukemogenic MCF MuLVs may reside in LTR and 3' pol sequences. (Theodore and Khan).

Mutagenesis of nucleotides encoding conserved amino acid residues in MoMuLV reverse transcriptase. Site specific mutagenesis was used to change two of four highly conserved amino acids in the reverse transcriptase of Moloney MuLV. Preliminary results with one of the mutants show that an asparagine to aspartic modification prevented development of infectious particles in cells transfected with cloned MoMuLV proviral DNA carrying this mutation. In vitro reverse transcriptase activity was also decreased two to three fold. This mutation therefore delineates a functionally important domain of reverse transcriptase. (Repaske).

Molecular analysis of a proviral insertion region in hematologic neoplasms induced by Friend MuLV. Two myeloid and three thymic lymphomas out of a total of 90 Friend (F) MuLV-induced tumors contained proviruses that had integrated within the same 2.5 kb region of cellular DNA. This region, designated Fis-1, mapped to the same place on mouse chromosome 7 as Int-2, a putative oncogene involved in murine mammary carcinomas. (This region may also be involved in human neoplasia since the human homologue of Int-2 maps to the same chromosome band as translocation breakpoint in human B cell lymphomas.) To investigate the relationship between Fis-1 and Int-2 approximately 30 kb of DNA surrounding Fis-1 was cloned from a mouse DNA library. No overlap was found between this region and 30 kb of DNA surrounding Int-2. Analysis of mRNA from a limited number of normal tissues and tumors with and without F-MuLV insertions in Fis-1 revealed no expression of sequences homologous to the cloned region of Fis-1. Furthermore, provirus insertion in Fis-1 does not induce expression of Int-2 mRNA characteristic of MMTV-induced mammary carcinomas. Studies in are progress to ascertain whether the region of cloned DNA surrounding Fis-1 contains a new oncogene activated by F-MuLV provirus insertion. (Silver and Buckler)

Characterization of DNA sequences homologous to prototype human endogenous retrovirus 4-1. Human chromosomal DNA contains endogenous retroviral sequences that are evolutionarily related to C-type mammalian retroviruses. One class of these sequences (the 4-1 family) has between 35 and 50 members and contains gag, pol and env sequences that are homologous to analogous genes in MuLVs. During the past year a second class (NP) of endogenous sequences was identified and cloned from a human gene library. NP retroviral segments contain gag, pol, env and LTR components: only 2 or 3 copies of NP sequences are present in the human gene. Two of the copies are present in the DNA of males but not females, which implies that they on the Y chromosome. Some females lack NP env sequences altogether, while other females (and some males) inherit a third form of the NP-env gene which identifies a novel class of endogenous human retrovirus. Studies are underway to assess when, in evolutionary terms, the NP env gene entered the human genome, and whether the presence of copies of this gene on the Y chromosome has biologic significance. (Silver, Rabson, Martin)

Genetic mapping of chromosomal genes involved in viral oncogenesis. Hamster x mouse somatic cell hybrids and genetic crosses were analyzed to chromosomally localize proviral genes, cellular oncogenes and tumor-specific integration sites. A mouse mammary tumor (MMTV) provirus of BALB/c was mapped to chromosome 6 near the immunoglobulin gene and non-germline copies found in tumors and established tumor lines were mapped to chromosomes 3 and 17. The cellular homologs of the Rel and ErbB oncogenes were mapped to chromosome 11, and it was shown that sequences homologous to the 3' and 5' ends of v-ets are on 2 different mouse chromosomes. Tumor-specific integration sites for MuLVs were mapped to chromosomes 7 and 15, and interferon genes were mapped to chromosomes 4, 12, and X. (Kozak, Callahan, Jolicoeur, O'Brien, Silver, Pitha, Dudley).

Ras amplification and malignant transformation. It has been previously shown that transfection of multiple copies of the human H-ras proto-oncogene induces malignant transformation of NIH-3T3 cells. During the past year an amplification of this locus in unmanipulated human tumor biopsies has been observed, although at a relatively low frequency. These results indicate that ras gene amplification is not a consequence of in vitro establishment of cell lines and indeed may constitute an alternative pathway through which ras can also contribute to neoplastic development. (Santos and Barbacid)

Monoclonal antibodies against p21 proteins. Using highly purified, bacterially synthesized, transforming and normal p21 proteins as antigens, a panel of monoclonal antibodies has been obtained. These antibodies are being characterized in terms of differential affinity for both forms of the protein and of their specific effect on the known biochemical properties of these proteins. The antibodies will be useful reagents for the functional mapping of ras oncogenes in pathological specimens. (Bryan, Santos).

Characterization of genes controlling cell proliferation in lower eukaryotes. Lower eukaryotic systems amenable to simple genetic and biochemical analysis such as fungi and *Drosophila* have been used to define the function of oncogenes and other cellular determinants involved in cell proliferation and differentiation. A locus (*Flu1*) has been identified in *Aspergillus nidulans*, that is activated by 5-azacytidine and results in uncontrolled fungal proliferation. This locus is presently being molecularly cloned and its evolutionary analogues in higher eukaryotes will be ascertained. (Tamame, Bryan, Santos)

A comparative analysis of multiple antibiotic resistance plasmids from group D streptococci of human and animal origin. Preliminary evidence has been obtained for the presence of the Em, Km and Sm resistance determinants of pJH1, and one other plasmid containing the same resistance genes, on an 18 to 22 kbp transposon. A 58 kb R plasmid, pLDR517, from a strain of *S. faecalis* of animal origin, also mediating resistance to Em, Km, and Sm, exhibited no detectable homology to the cloned resistance genes of pJH1. The resistance genes are currently being cloned from pLDR517 and will be used as hybridization probes to assess the extent to which they have been disseminated among streptococci of human and animal origin, and whether they may also be present in strains previously shown to harbor the pJH1-related resistance determinants. (LeBlanc and Lee)

Characterization of a novel spectinomycin resistance determinant in *Streptococcus faecalis*. A gene encoding AAD activity, which adenylates spectinomycin, but not streptomycin, was cloned on a 1.1 kpb *Cla*I-*Nde*I fragment in *Streptococcus sanguis* and *E. coli*, and expressed resistance to spectinomycin at 10,000 and 50,000 µg/ml, respectively. The cloned fragment was sequenced and shown to contain a 783 bp open reading frame encoding a protein with a predicted size of 28,943 daltons. This gene shared nearly 50% polynucleotide sequence homology with a similar gene from a strain of *Staphylococcus aureus*. The predicted amino acid sequences of the two genes exhibited between 40 and 50% homology. The streptococcal and staphylococcal genes are currently being used as probes in hybridization reactions with purified DNA from *Nisseria gonorrhoeae* isolates exhibiting high level resistance to spectinomycin. (LeBlanc and Lee)

Plasmid-mediated lactose metabolism in group N streptococci. Restriction endonuclease fragments from the *Streptococcus sanguis* chromosome that contain the site of insertion of lactose-specific DNA sequences from a *Streptococcus cremoris* plasmid have been identified and are being molecularly cloned in an *E. coli* host/vector system. Chromosomal DNA from a *lac*⁻ strain of *Streptococcus faecalis* was also shown to share extensive homology with cloned lactose-specific phosphoenolpyruvate-dependent phosphotransferase and phospho-β-galactosidase genes from the *S. cremoris* plasmid. (LeBlanc and Lee)

Molecular and genetic analysis of plasmid pAMB1. More than 150 random and overlapping fragments, with an average size of 600 bp, have been cloned into phage M13 DNA from a high copy number broad host-range replicon originally derived from pAMB1. These clones are currently being used for the sequencing of this replicon. A protoplast transformation system, using a *S. faecalis* host strain, has been adapted for the cloning of a second, low copy number narrow host-range replicon, also suspected to be present in pAMB1. (LeBlanc and Lee)

Molecular studies of the pBI136 *Cln*^r gene. Previous work has shown that the clindamycin resistance (*Cln*^r) determinants on *Bacteroides* R-plasmids are part of transposon-like structures bounded by 1.2 kb direct repeat sequences (DRS). Cloning experiments and DNA sequence analysis of the *Cln*^r gene from pBI136 showed that the gene started 17 bp from the DRS terminus and that the promoter for the determinant is found within the DRS. These results provide insight on how *Bacteriodes* spp. regulate expression of newly acquired genetic information. In addition, information obtained concerning the structure of the *Cln*^r gene enabled the construction of plasmid vectors suitable for the determination of promoter activity in cloned segments of *Bacteriodes* DNA. (Smith).

Genetic analysis of transposition of the pBI135 Cln^r determinant. A model system was designed to demonstrate transposition of the pBI136 Cln^r determinant in Bacterioides fragilis. A region of pBI136 DNA encompassing the entire 8.4 kb transposon-like structure was cloned on a plasmid vector defective for replication in Bacterioides. The resulting plasmid, pFD197, was transformed into Bacterioides and clones resistant to clindamycin were screened for the presence of pFD197. (Smith)

Non-plasmid associated genetic exchange in Bacterioides. The conjugal transfer of non-plasmid associated antibiotic resistance determinants is an important means of genetic exchange among Bacterioides spp. These novel transfer systems can also mobilize small non-conjugative plasmids and this feature has been studied using a variety of recombinant plasmids. Results thus far suggest that there are important host encoded functions responsible for the transfer event and that these may not be linked to the antibiotic resistance determinants as previously thought. Conjugal transfer from Bacterioides to E. coli also was demonstrated and this will open new avenues of investigation. The next step in understanding these transfer systems is to identify regions of the mobilized plasmids required for their transfer and this is currently being studied using plasmid deletions and DNA sequencing. (Smith).

Attachment moiety and pathogenicity of mycoplasma genitalium. A specific 143 kd protein has been identified as the principal immunodominant component of this organism and is involved in its adherence to tissue components. The protein is surface accessible, trypsin-insensitive, and is clearly distinct from the 165 kd P1 protein, identified as the principal attachment component in pathogenic strains of M. pneumoniae. A monoclonal antibody prepared to the 143 kd protein was employed in a radioimmunoprecipitation assay to demonstrate the uniqueness of this determinant to M. genitalium. (Tully and Baseman)

Biological and molecular features of acholeplasmas and spiroplasmas. A cluster of 12 sterol non-requiring acholeplasmas isolated from a variety of human and non-human sources were compared biochemically and serologically. Most isolates were found to be unrelated to known Acholeplasma species yet possessed biological features of the group including genome sizes of about 1000 Md and a G+C ratio of 27-31 mol %. Approximately 50 helical mycoplasmas (spiroplasmas), primarily from plant and insect habitats, have been assigned to about 30 distinct groups, based upon analysis of their serological activities, genome size (also 1000 Md), base composition (23 to 30 mol % G+C), restriction endonuclease patterns, polyacrylamide gel patterns of cell proteins, and DNA hybridization properties. (Tully)

Characterization of an mRNA transcript encoding the "A" open reading frame of the AIDS retrovirus. The "A" open reading frame of the AIDS retrovirus (RV) is expressed as two subgenomic RNA species of 5.5 and 5.0 kb. A partial cDNA clone of the 5' end of the 5.0 kb RNA has been isolated and shown by DNA sequence analysis to encode a spliced mRNA containing the entire A gene. By coupled in vitro transcription with SP6 polymerase and translation in a rabbit reticulocyte lysate, the A cDNA has been shown to encode a 23 kd protein product, the same size as the authentic A protein present in virally infected cells. The function of the A protein is currently being analyzed by mutagenesis of infectious AIDS RV DNA. Deletion of the C-terminal portion of A results in a retardation of viral infectivity. (Daugherty, Rabson, Martin)

The construction of an infectious molecular clone of the AIDS virus. An infectious clone of the AIDS virus has been constructed by ligating the 5' and 3' halves of two integrated proviruses together at the EcoRI site located at 5.7 kb. Upon transfection, this clone directed the production of infectious virus particles in a wide variety of cells in addition to human T4 cells. Infectious progeny virions were synthesized in mouse, mink, monkey and several human non-T cell lines indicating the absence of any intracellular obstacle to viral RNA or protein production or assembly. During the course of these studies, a human colon carcinoma cell line, 8E5, exquisitely sensitive to DNA transfection, was identified. (Adachi, Gendelman, Rabson, Willey, Martin)

The identification of conserved and divergent domains within the envelope gene of the AIDS retrovirus. The nucleotide sequences of the envelope genes of an African and a North American AIDS viral isolate have been determined. When their deduced amino acid sequences were aligned with the envelopes of the LAV and ARV isolates, conserved and divergent regions were readily identified. Hypervariable stretches of 28 to 74 amino acids, exhibiting 20 to 30% amino acid identity at each position and characterized by reciprocal insertions and deletions, were confined to the gp120 external envelope protein. These variable domains abutted 4 regions of highly conserved (among the 4 isolates) amino acids. In vitro mutagenesis is being employed to evaluate the origin and functional significance of the unstable envelope gene of the AIDS virus (Willey, Rutledge, Theodore, Buckler, Martin)

Productive, persistent infection of human colorectal cell lines with the AIDS retrovirus. Thirteen adherent human non-lymphocyte cell lines were tested for their susceptibility to infection by the AIDS retrovirus. Productive infection could be demonstrated in 3 of 5 colorectal carcinoma cell lines examined; the other 8 human non-lymphocyte cell lines were uninfected. A susceptible colon carcinoma cell line as well as normal colon cells were shown to actively synthesize polyadenylated CD4 RNA whereas uninfected colon and rhabdomyosarcoma cell lines contained no detectable T4 RNA. A persistently infected colon carcinoma cell line was established that continued to produce progeny virus for more than 10 weeks post-infection. (Adachi, Koenig, Gendelman, Daugherty, Gattoni-Celli, Fauci, Martin).

Structural characterization of the reverse transcriptase and endonuclease polypeptides of the AIDS retrovirus. Automated N-terminal microsequencing of immune affinity purified AIDS retrovirus polypeptides from infected cells was utilized to locate the N-termini of 64, 51 and 34 kD polypeptides within the *pol* reading frame of the proviral DNA. The 64 and 51 kD proteins had identical N-termini (ProIleSerProIleGluThrVal---) positioned 156 residues from the beginning of the *pol* open reading frame (ORF). The N-terminus of the 34 kD *pol* gene product, PheLeuAsnGlyIleAspLys---, mapped 716 residues into the *pol* ORF. These polypeptides were absent in a RT negative, CD4 negative cell line (8E5) carrying a single defective copy of a constitutively expressed integrated proviral DNA. Pulse-chase labeling studies are presently in progress to determine the processing of the *gag* and the *gag-pol* precursor proteins and to dissect the molecular lesion in RT negative viral mutants. Other viral proteins, notably a 17 kD putative protease and two novel 41 kD virus coded proteins are also being sequenced. (Venkatesan, Coligan, Lightfoote and Mervis)

Identification of the target cells for the AIDS virus in the central nervous system. Premortem neurological findings are detectable in approximately one-third of AIDS patients while neuropathological changes are present in more than three-quarters of autopsied subjects. Using *in situ* hybridization coupled with immunocyto-chemical techniques, mononucleated and multinucleated macrophages actively synthesize viral RNA and produce progeny virions in the brains of patients with AIDS and ARC. The infected brain macrophages were shown to may arise from circulating blood monocytes and thereby serve both as a reservoir and vehicle for viral dissemination in the infected host (Koenig, Gendelman, Martin, Fauci).

The molecular characterization of an AIDS provirus containing a mutation in the polymerase gene. An AIDS provirus that is unable to synthesize the reverse transcriptase and endonuclease polypeptides and produces non-infectious viral particles has recently been described. A full-length molecular clone of the provirus (p8E5) was obtained and its biologic activity assayed by transfection. The mutation in p8E5 DNA was mapped to a portion of the polymerase gene located from bp 2630 to bp 4562 in the AIDS proviral DNA. The nucleotide sequence of this fragment revealed several base substitutions resulting in eight amino acid changes compared to other infectious proviral DNAs. This finding indicates that base substitutions, in the absence of termination codon(s) and deletions result in produce defective polymerase gene products and prevent the synthesis of infectious virus particles. (Gendelman and Martin)

Structural analysis of an endogenous MCF-related mRNA. MCF MuLVs have been implicated as the proximal causal agent in case of AKR thymic lymphomas. These viruses arise de novo by recombination between ecotropic, xenotropic and endogenous MCF-related sequences. Prior to the appearance of full-length 8.4 kb MCF viral mRNAs, 7.2 kb, 3.0 kb and 1.8 kb transcripts were detected in 1 to 2 month old AKR mouse thymus tissue. To study the structure of potential recombinational partners in MCF virus genesis, a 2.0 kb cDNA segment of the 7.2 kb mRNA species was cloned and its nucleotide sequence determined. Sequence analysis indicated a 1.2 kb deletion in the env region. Since the cDNA was closely related to a previously cloned endogenous MCF-related provirus which contained an identical env deletion, the latter DNA family may serve as templates to possible precursors of MCF viruses. (Khan, Repaske, Amanuna).

Characterization of two novel endogenous MuLV-related gene families. Two unique retroviral DNAs, B-26 and B-60, were cloned from the BALB/c mouse genome. The DNAs were distantly related to each other and known infectious MuLV proviruses in the gag, pol and env regions. Nucleotide sequence analysis of the 3' proviral termini indicated that B-60 and B-26 DNAs contained different retroviral-like LTRs distinct from MuLV LTRs. Comparative distribution studies of B-26 and B-60-related proviruses and infectious MuLV sequences in a series of mouse, rat and Chinese hamster DNAs indicated that B-26 and B-60 gene families were ancient and had amplified in the mouse genome. (Khan and Obata)

Honors and Awards

Malcolm Martin

Invited speaker: The Johns Hopkins University School of Medicine, Baltimore, Maryland; January, 1986.

Invited speaker: Cologne Spring Meeting, Cologne, West Germany; February, 1986.

Organize and Session Chairman: Wallace Rowe Symposium on Animal Viruses, Bethesda, Maryland; February, 1986.

Invited speaker: Washington University, St. Louis, Missouri; March, 1986.

Invited speaker: Worcester Foundation for Experimental Biology, Worcester, Massachusetts; April, 1986.

Organizing Committee and Session Chairman: Second International AIDS Conference, Paris, France; June, 1986.

Invited speaker: Cold Spring Harbor Conference, "Modern Approaches to Vaccines," Cold Spring Harbor, New York; September, 1986.

Invited speaker: Molecular Biology and Cancer Conference, Santander, Spain; September, 1986.

Scientific Review Board: Lucille P. Markey Charitable Trust, Miami, Florida.

Donald LeBlanc

Convenor: Seminar on "Dissemination of Tetracycline Resistance Determinants into Mycoplasmas and Gram-positive and Gram-negative Bacteria," 86th Annual Meeting of the American Society for Microbiology, Washington, DC; March, 1986.

Invited speaker: AOAC Spring Training Workshop, Seattle, Washington. "Studies on the Dissemination of Streptococcal Antibiotic Resistance Determinants in the Natural Environment"; April, 1986.

Invited speaker: ASM Conference on Streptococcal Genetics, Miami, Florida "Comparative Analysis of R Plasmids from Streptococci of Human and Animal Origin"; May, 1986.

C. Jeffrey Smith

Invited lecturer: Virginia Commonwealth University, Richmond, Virginia; October, 1985.

Editorial Board, Applied and Environmental Microbiology

Eugenio Santos

Severo Ochoa Award for Biomedical Research; Barcelona Spain; January, 1986.

Invited speaker: Symposium on Drugs and Cancer; Madrid, Spain; September, 1985.

Invited speaker: University of Barcelona, Spain; January 1986.

Joseph Tully

Invited speaker: International Symposium on Ureaplasmas of Humans; Seattle, Washington; October, 1985.

Invited speaker: University of Texas, Health Science Centre, San Antonio, Texas; December, 1985.

Co-convenor and speaker: Seminar on "Spiroplasmas: Recent Developments in the Biology of Helical, Wall-less Mollicutes," American Society for Microbiology Meeting, Washington, DC; March, 1986.

Associate Editor: International Journal of Systematic Bacteriology.

Chairman, J. Porter Award Committee, American Society for Microbiology.

Arnold Rabson

Invited speaker: Inter-American Society of Chemotherapy, Tampa, Florida; December, 1985.

Invited speaker: George Washington University, Georgetown University, Washington, DC; March, 1986.

Invited speaker: Lombardi Cancer Center, Georgetown University, Washington, DC; April, 1986.

Invited speaker: FASEB Summer Research Conference on Autoimmunity, Saytons River, Vermont; June, 1986.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00027-19 IMM
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Basic Studies of Mycoplasmas		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Joseph G. Tully	Head, Mycoplasma Section IMM, NIAID
Others:	David L. Rose	Research Microbiologist IMM, NIAID
COOPERATING UNITS (if any) J. B. Baseman, University of Texas, San Antonio J. M. Bove, University of Bordeaux, France R. F. Whitcomb, USDA, Beltsville, MD D. Taylor Robinson, Clinical Res. Centre, Harrow, UK		
LAB/BRANCH Laboratory of Molecular Microbiology		
SECTION Mycoplasma Section		
INSTITUTE AND LOCATION NIAID, NIH, Frederick, Maryland 21701		
TOTAL MAN-YEARS: 3.0	PROFESSIONAL: 1.0	OTHER: 2.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Recent efforts involve further characterization of a newly discovered mycoplasma (<u>Mycoplasma genitalium</u>) in the urogenital tract of patients with non-gonococcal urethritis. The organism shares some partial antigenic relationship to another pathogenic mycoplasma of man (<u>M. pneumoniae</u>), and this relationship is thought to be mediated by similarities in surface components on the unique terminal attachment structure found in both organisms. Polyclonal and monoclonal antibodies to the P1 attachment protein (165 kd) in virulent <u>M. pneumoniae</u> strains did not react with whole cell or soluble preparations from <u>M. genitalium</u>, indicating the latter organism had a distinct attachment moiety. A specific 143 kd protein has been identified as the possible component in the adherence of <u>M. genitalium</u> to tissue cells. Antibody to both the 165 kd P1 protein and the 143 kd protein have been shown to appear in the serum of animals recovering from experimental infections with each of the two established species. Experimental primate infections with <u>M. genitalium</u> indicated the organism is capable of colonizing the male and female urogenital tract, and in some cases invaded the circulatory system.</p> <p>In more recent collaborative studies, clinical and laboratory evaluation of a case of acute arthritis in a 34 year-old female provides fairly strong circumstantial evidence that <u>M. genitalium</u> might also be involved in extra-genital tract disease. Studies on other mollicutes (non-sterol requiring achleplasmas and helical spiroplasmas) have centered around biochemical and molecular features of these organisms and their possible involvement in pathological syndromes in humans.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 0000190-08
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) The Molecular Genetics of Eukaryotic Cells and Their Viruses		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Malcolm A. Martin	Chief LMM, NIAID
Others:	Akio Adachi	Visiting Fellow LMM, NIAID
	Howard Gendelman	Expert LMM, NIAID
	Theodore Theodore	Research Microbiologist LMM, NIAID
COOPERATING UNITS (If any)		
	Thomas Folks	Staff Fellow LIR, NIAID
	Marilyn Lightfoote	Staff Fellow LIR, NIAID
LAB/BRANCH Laboratory of Molecular Microbiology		
SECTION Biochemical Virology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 3	PROFESSIONAL: 1	OTHER: 2
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> Infection of human T lymphocytes or T lymphocyte lines with the AIDS retrovirus (RV) can result in a variety of outcomes ranging from rapid cell death to the stable integration of functionally inert proviral DNA and no obvious viral cytopathic effect. A typical AIDS RV infection of CD4⁺ lymphocytes is characterized by the rapid appearance of multinucleated cells, a burst of reverse transcriptase activity and profound cellular degeneration that extends over a 5 to 20 day period. During the past year we have characterized CD4⁺ cells that survive acute infection and which neither produce nor are infectable by the AIDS RV. Such "survivor" cells can be induced to produce infectious virus following treatment with 5-iodo-2'-deoxyuridine (IUdR). A cellular clone was isolated from a mass culture of survivor cells that contained a single copy of the AIDS RV provirus. The integrated proviral DNA was constitutively expressed but generated defective virus particles that failed to synthesize reverse transcriptase. The biological and biochemical properties of this cloned survivor cell has been investigated with the goal of understanding the interaction of viral gene products involved in cell killing. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00218-05 LMM
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Biochemical and Chemical Studies on Retroviral DNA (revised title)		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Roy Repaske	Research Chemist LMM, NIAID
Others:	Raymond O'Neill	Chemist LMM, NIAID
	Arifa Khan	Senior Staff Fellow LMM, NIAID
	Christine Kozak	Microbiologist LMM, NIAID
	Janet Hartley	Research Microbiologist LVD, NIAID
	Malcolm Martin	Chief LMM, NIAID
	Arnold Rabson	Medical Staff Fellow LMM, NIAID
COOPERATING UNITS (if any) <div style="text-align: center; padding-top: 10px;">None</div>		
LAB/BRANCH Laboratory of Molecular Microbiology		
SECTION Biochemical Virology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205		
TOTAL MAN-YEARS: 2	PROFESSIONAL: 1	OTHER: 1
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unabbreviated type. Do not exceed the space provided.) <p>A. Alignment of deduced amino acid sequences of the <u>pol</u> gene of human endogenous C-type proviral DNA and corresponding sequences from a variety of retroviruses found in man (HTLV-I, -II, -III), ape, cow, sheep, mouse and chicken shows little homology in the region encoding reverse transcriptase even though the enzyme performs the same function in all retroviruses. A highly conserved small domain was identified in reverse transcriptases as well as in transposons and other sequences attributed to having reverse transcriptase function. Moloney murine leukemia virus (Mo-MuLV) is being used as a model system to evaluate the significance of these conserved sequences. Site specific mutagenesis has been used to change the codons of the conserved amino acids. The normal sequence has been replaced in MoMuLV and in a reverse transcriptase-containing expression vector to permit evaluation of these mutations biologically in infected cells and biochemically in an <u>in vitro</u> system, respectively.</p> <p>B. Of the four C-type murine retroviruses known, three (ecotropic, xenotropic and MCF MuLVs) can exist as integrated sequences in mouse genomic DNA; definitive results had not been obtained for the fourth type, amphotropic MuLV. It has not been possible to determine number of copies or types of each of the MuLVs or to follow the genesis of MCF MuLV (which is associated with leukemia in mice) since a specific hybridization probe was available only for ecotropic MuLVs. We have developed individual probes for MCF, xenotropic and amphotropic MuLVs which hybridize specifically with the <u>env</u> region of the respective MuLV. Analysis of genomic DNA of inbred laboratory mice show all mice have many copies of MCF and xenotropic sequences with more copies of the former. Wild mice, on the other hand, may have neither sequence of both sequences. These data indicate mouse speciation occurred before MCF or xenotropic MuLVs were integrated into the germline. No mice have amphotropic sequences suggesting they represent an exogenous rather than an endogenous type virus.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00219-05 LMM
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Molecular and Genetic Analysis of Streptococci		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: Donald J. LeBlanc Head, BVS LMM, NIAID Others: Linda N. Lee Chemist LMM, NIAID		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Molecular Microbiology		
SECTION Bacterial Virulence Section		
INSTITUTE AND LOCATION NIAID, NIH, Frederick, Maryland 21701-1013		
TOTAL MAN-YEARS 2	PROFESSIONAL 1	OTHER 1
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)		
<p> Southern blot hybridizations were used to identify restriction endonuclease fragments from <u>Streptococcus sanguis</u> Challis chromosomal DNA that contain the site of insertion of lactose-specific sequences from cloned fragments of a <u>Streptococcus cremoris</u> lactose metabolic plasmid. The fragments, which include a 15 kilobase pair (kbp) <u>EcoRI</u> fragment, a 6.0 kbp <u>HincII</u> fragment, a 3.2 kbp <u>AccI</u> fragment, a 20 kbp <u>BamHI</u> fragment, a 23 kbp <u>PstI</u> fragment, and two <u>HindIII</u> fragments of 5.0 and 2.5 kbp, are currently being cloned in <u>E. coli</u> to 1) examine their relationship to the lactose-specific sequences, and 2) to replace their regions of homology to the plasmid DNA with a kanamycin resistance gene. The resultant fragments will be excised from the <u>E. coli</u> vector and used to transform lactose <u>S. sanguis</u> strains with selection for kanamycin resistance. Residual homology to the <u>S. sanguis</u> chromosome should permit recombination and replacement of the sequences exhibiting homology to the lactose plasmid. It is expected that hybrid plasmids containing lactose-specific genes will be stably maintained in such a strain, thus permitting further studies on the expression of these lactose genes in a streptococcal host. Chromosomal DNA from a lac <u>Streptococcus faecalis</u> strain was also shown to share extensive homology with the cloned lactose PEP-dependent phosphotransferase and phospho-β-galactosidase genes from the <u>S. cremoris</u> plasmid. More than 150 random and overlapping fragments of a 5 kbp <u>EcoRI</u> fragment from pAMB1, containing the extensively studied broad host-range replicon from this plasmid, have been cloned onto an M13 vector in preparation for DNA sequencing. A <u>S. faecalis</u> protoplast transformation system is also being employed for the cloning of a second, narrow host-range replicon, unable to function in <u>S. sanguis</u>, whose presence in intact pAMB1 has been indicated by a series of incompatibility studies using previously obtained natural and subcloned derivatives of pAMB1. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00222-05 LMM
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Characterization of Endogenous Ecotropic and Xenotropic Murine Leukemia Virus		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Theodore Theodore	Research Microbiologist LMM, NIAID
Others:	Arifa S. Khan Malcolm A. Martin	Senior Staff Fellow Chief LMM, NIAID LMM, NIAID
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Molecular Microbiology		
SECTION Biochemical Virology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 1	PROFESSIONAL: 1	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> Mink cell focus forming (MCF) viruses are dualtropic murine leukemia viruses (MuLVs) which have been isolated from leukemic mouse tissue. These viruses have been divided into two classes based on their ability to accelerate the onset of thymic lymphomas in AKR mice. Both classes of MCF viruses arise by recombination involving ecotropic MuLV and endogenous MCF-related <i>env</i> sequences. Leukemogenic MCF MuLVs (class I) replicate efficiently in the thymus whereas the non-leukemogenic (class II) grow poorly in the thymus. Long terminal repeats (LTRs) have been shown to be involved in tissue-specificity and disease induction associated with leukemogenic MuLVs; also, sequences present in the <i>gag</i>, <i>pol</i>, or <i>env</i> regions have been shown to be necessary for expression of maximum leukemogenicity. To study the sequences of MCF-MuLVs involved in viral leukemogenesis, molecularly cloned DNAs of leukemogenic MCF-13 and non-leukemogenic MCF-111A MuLVs were obtained and the nucleotide sequences of the LTRs, 3' <i>pol</i> and 5' <i>env</i> regions determined. The results of comparative sequence analysis indicated differences in the LTR and 3' <i>pol</i> regions between MCF-13 and MCF-111A MuLV DNAs. The LTR associated with MCF-13 was closely related to that present in xenotropic MuLVs, whereas the LTR sequence of MCF-111A that identical except for 1 bp to the ecotropic proviral LTR. A 12 bp nucleotide stretch, characteristic of leukemogenic MCF MuLVs, was present in MCF-13 in the 3' <i>pol</i> region but was lacking in MCF-111A. No significant sequence divergence was seen between MCF-13 and MCF-111A MuLV DNAs in the <i>env</i> region. These results suggest that the leukemogenic potential of MCF-13 may reside in LTR and 3' <i>pol</i> sequences. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00281-05 LMM
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Molecular Cloning of Recombinant MuLV Proviral Sequences in Inbred Mice		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Charles E. Buckler Research Biologist	LMM, NIAID
Others:	Jon Silver Medical Officer	LMM, NIAID
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Molecular Microbiology		
SECTION Viral Biology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 0.5	PROFESSIONAL: 0	OTHER: 0.5
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The project has been extended to study the molecular nature of the mouse genome containing the genetic locus. <u>Fis-1</u>. This locus has been shown to be the site of integration of Friend MuLV in some lymphomas appearing after inoculation of several inbred mouse strains with the ecotropic Friend MuLV. In about 5% of approximately 100 tumors examined, copies of Friend MuLV were found integrated within a 1.5 kb region of the mouse genome (<u>Fis-1</u>). From a mouse genomic library constructed in lambda virus, 35 kb of mouse genomic sequence surrounding the <u>Fis-1</u> locus were isolated. These mouse sequences were present in two recombinant lambda phages. The mouse sequence inserts in the lambda phage were subcloned into plasmids and fragments from 7 to .3 kb were obtained. Each subcloned fragment was analyzed for unique or repetitive mouse sequence content by Southern blot analysis. Subclones containing a total of about 15 kb of unique mouse sequence were further utilized to test for expression in RNA isolated from mouse tumors. No RNA expression has been detected in normal mouse tissue or in mouse tumors (either with or without Friend MuLV insertions in the <u>Fis-1</u> region). Sequence determination of the 1.5 kb <u>Fis-1</u> sequence is in progress. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00300-05 LMM
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Control of Resistance to Viral Leukemogenesis in Wild Mouse Populations		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Christine A. Kozak Microbiologist	LMM, NIAID
Others:	Ray O'Neill Chemist Peter Voytek Guest Worker	LMM, NIAID LMM, NIAID
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Molecular Microbiology		
SECTION Viral Biology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	1	1
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Studies on viral leukemogenesis in wild mice have shown that these mice differ substantially from the inbred strains. First, feral mice contain many fewer chromosomally integrated MuLV related sequences than inbred mice. Second, the ecotropic virus isolates from <i>M. hortulanus</i> , <i>M. m. castaneus</i> , and <i>M. cervicolor</i> differ from the ecotropic MuLVs of inbred mice in their biochemical and biological properties. Third, cells of most wild mice differ from inbred mice in their susceptibility to exogenous infection by ecotropic and xenotropic MuLVs. Most feral mice carry a novel non-restrictive allele at the <i>Fv-1</i> locus and some Maryland wild mice show an unusual resistance to NB-tropic virus. Cells of most feral mice, but not laboratory mice, are susceptible to xenotropic MuLVs. This trait is controlled by a single chromosome 1 locus, designated <i>Sxv</i> which may represent a wild mouse polymorphism of the MCF-MuLV receptor locus.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00301-05 LMM
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Cellular Genes Involved in Transformation and MuLV Transmission in Inbred Mice		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Christine A. Kozak Microbiologist	LMM, NIAID
Others:	Robert Callahan Daniel Nebert Jonathan Silver Stephen O'Brien	LTIB, NIAID LDP, NIAID LMM, NIAID LVC, NCI
COOPERATING UNITS (if any)		
P. Jolicoeur M. Proffitt C. Croce	Clinical Research Institute of Montreal, Canada Cleveland Clinic Foundation, Ohio Wistar Institute, Philadelphia, Pa.	
LAB/BRANCH Laboratory of Molecular Microbiology		
SECTION Viral Biology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205		
TOTAL MAN-YEARS: <div style="text-align: center;">2</div>	PROFESSIONAL: <div style="text-align: center;">1</div>	OTHER: <div style="text-align: center;">1</div>
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Genetic studies on the transmission of murine retroviruses through the mouse germline, susceptibility to exogenous infection, and virus-induced oncogenesis have led to the identification and chromosomal mapping of numerous genetic loci involved in these phenomena. These studies rely on the analysis of somatic cell hybrids either alone or in conjunction with classical Mendelian crosses. The genes under investigation include proviral integration sites for various mammary tumor viruses and MuLVs, the cellular homologs of several oncogenes, and tumor-associated integration sites. Hybrids have also been used to map several other genes for which cloned probes are available including interferon and α -, β -spectrin.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 AI 00304-05 LMM
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Pathogenesis of Disease Induced by Friend MuLV		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Jonathan Silver	Medical Officer LMM, NIAID
Others:	Charles E. Buckler	Research Biologist LMM, NIAID
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Molecular Microbiology		
SECTION Viral Biology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1	0.5
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.) <p> F-MuLV can induce several different hematopoietic neoplasms including T-+ B-Cell lymphoma, erythroleukemia and myeloid leukemia. As one approach to studying disease pathogenesis in this system, we cloned sites of F-MuLV provirus insertion in host DNA in an F-MuLV-induced myeloid leukemia. One 2.5 kb region identified in this manner was found to contain an F-MuLV provirus in one other myeloid leukemia and three thymic lymphomas out of a total of 90 tumors examined. This result indicated that proviral insertion sites in F-MuLV-induced tumors in host DNA are not random. It is likely that provirus insertions in this common integration region, designated <u>Fis-1</u>, confers a growth advantage. We mapped the <u>Fis-1</u> locus to mouse chromosome 7, about 26 cM distal to the locus for beta globin. Surprisingly, we found that <u>Fis-1</u> mapped to the same position as <u>Int-2</u>, a putative oncogene involved in murine mammary carcinomas. Nevertheless, <u>Fis-1</u> and <u>Int-2</u> are distinct since 30 kb of DNA surrounding <u>Fis-1</u> do not overlap 30 kb surrounding <u>Int-2</u>, and proviral insertions in <u>Fis-1</u> do not induce <u>Int-2</u> mRNA. This <u>Fis-1/Int-2</u> region may be involved in human B cell neoplasm since the human homologue of <u>Int-2</u> has been mapped to the same chromosomal band as a locus involved in human B cell lymphoma, <u>Bcl-1</u>. Taken together, these observations identify a chromosomal region involved in the pathogenesis of a wide variety of malignancies in mouse and man. We are currently extending the region of cloned DNA surrounding <u>Fis-1</u> in the hopes of finding an oncogene(s) which is activated by F-MuLV insertion and highly conserved between mouse and man. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00353-04 LMM
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Structural and Functional Analysis of Endogenous Proviruses of Mice (Revised Title)		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Arifa S. Khan Senior Staff Fellow LMM, NIAID Others: Janet Hartley Chief, Viral Oncology Section LVD, NIAID Masahiro Obata Visiting Associate LMM, NIAID Theodore Theodore Research Microbiologist LMM, NIAID Roy Repaske Research Chemist LMM, NIAID		
COOPERATING UNITS (if any) Hiroshi Amanuma Scientist Riken, JAPAN		
LAB/BRANCH Laboratory of Molecular Microbiology		
SECTION Biochemical Virology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3	2	1
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Mouse genome contains at least 50 copies of murine leukemia viral (MuLV)-related DNAs, the majority of which are defective. Recombination between sequences of different infectious and defective MuLV DNAs results in the generation of novel MuLVs including lymphomagenic mink cell focus-forming (MCF) viruses. The main goals of this project are: 1) to determine the relatedness between endogenous MuLV sequences and infectious MuLVs; 2) to study generation of recombinant MCF viruses; and 3) to identify sequences contributing to leukemogenicity of MCF MuLVs.</p> <p>Two unique retroviral DNAs, B-26 and B-60, were cloned from the BALB/c mouse genome. The DNAs were distantly related to each other and known infectious MuLV proviruses in the <u>gag</u>, <u>pol</u> and <u>env</u> regions. Nucleotide sequence analysis indicated that B-26 and B-60 DNAs contained different retroviral-like LTRs, distinct from MuLV LTRs. Comparative distribution studies of B-26- and B-60-related sequences and infectious MuLV proviruses in a series of rodent DNAs indicated that B-26 and B-60 gene families were ancient and had amplified in the mouse genome.</p> <p>To study the structure of potential recombinational partners in MCF virus genesis, a 2.0 kb cDNA segment of endogenous MCF <u>env</u>-related 7.2 kb mRNA species was cloned and its nucleotide sequence determined. Sequence analysis indicated a 1.2 kb deletion in the <u>env</u> region. The cDNA was found to be closely related to a previously cloned endogenous MCF-related provirus which contained an identical <u>env</u> deletion, thus indicating that the latter DNA family might serve as templates to possible precursors of MCF viruses.</p> <p>Leukemogenic MCF MuLVs contain a unique 12 bp sequence in the integrase-coding region of the <u>pol</u> gene. To study the role of this nucleotide stretch in leukemogenesis the 12 bp were deleted by oligonucleotide mutagenesis from MCF13 proviral DNA. The virus obtained from transfection studies using the mutant DNA has been injected into young AKR mice to test whether the MCF viral genome retains its leukemogenic potential in the absence of the 12 bp sequence.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00388-03 LMM
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Functional Analysis of Human Retroviral Genes		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: Arnold Rabson Daryl Daugherty Akio Adachi Malcolm Martin Thomas Folks	Medical Staff Fellow Medical Staff Fellow Visiting Fellow Chief Expert	LMM, NIAID LMM, NIAID LMM, NIAID LMM, NIAID LIR, NIAID
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Molecular Microbiology		
SECTION Biochemical Virology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205		
TOTAL MAN-YEARS 1.5	PROFESSIONAL: 1	OTHER: 0.5
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The primary goals of this project are analysis of the controls of gene expression and viral replication of human retroviral genomes and study of the functions of their individual gene products. Over the past year, our studies have focused primarily on the molecular biology of the AIDS retrovirus (RV) although a few studies on human endogenous retroviral DNA segments have continued.</p> <p>Previously, we had defined the major classes of AIDS RV RNA species detected in infected cells. To more clearly define the nature of the transcripts capable of encoding the A open reading frame, we have isolated a cDNA clone derived from the 5.0 kb viral mRNA species. This clone is capable of directing the synthesis of a 23 kD "A" protein <u>in vitro</u>. Furthermore, the A gene product appears to be important for replication as deletion of this region from an infectious proviral clone diminishes the infectivity of that DNA.</p> <p>The function of different gene regions of the AIDS RV is being analyzed by insertion of these gene regions into eukaryotic expression vectors. The AIDS RV <u>tat</u> gene has been inserted into MuLV and SV-40 based vectors, the A cDNA into an SV40 vector, and the <u>env</u> gene is currently being placed into amphotropic MuLV. In addition, a cDNA clone for CD4, the AIDS RV receptor, has been cloned in an MuLV based expression system and will be used to study the mechanism of AIDS RV infection of human cell lines.</p> <p>In efforts to identify homologues of the AIDS RV in normal human cells, we have detected the presence of several DNA species in normal human DNA that hybridize to the <u>tat</u> region of the AIDS RV under low stringency hybridization conditions. These DNA sequences will be molecularly cloned and analyzed to determine their relationship to the AIDS RV.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00395-03 LMM
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Genetic and Molecular Analysis of Anaerobic Bacteria Indigenous to Humans		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: C. Jeffrey Smith Staff Fellow LMM, NIAID		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Molecular Microbiology		
SECTION Bacterial Virulence		
INSTITUTE AND LOCATION NIAID, NIH, Frederick, Maryland 21701		
TOTAL MAN-YEARS: 1.5	PROFESSIONAL: 1	OTHER: 0.5
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The clinically significant clindamycin resistance (Cln^r) plasmids were used as model systems to study genetic exchange mechanisms and gene expression in <u>Bacteriodes fragilis</u>.</p> <p>Previous work with these plasmids suggested that the Cln^r determinants were located on transposon-like structures bounded by 1.2 kb directly repeated sequences. DNA sequence analysis of the resistance gene from one of these plasmids, pBI136, showed that it started just 17 bp from the terminus of one of the repeated sequences. There were no promoter-like sequences associated with the gene and cloning experiments indicated that sequences upstream, from within the direct repeat, were required for expression of Cln^r in <u>Bacteriodes</u>. Cloning vectors containing the Cln^r gene without its promoter sequences were constructed and used to demonstrate the presence of promoter activity in both copies of the direct repeat sequence.</p> <p>Transposition of the pBI136 Cln^r determinant in <u>Bacteriodes</u> was demonstrated by cloning the entire transposon-like structure onto a shuttle plasmid vector defective for replication in <u>Bacteriodes</u>. Following transformation with this plasmid, clindamycin resistant clones of <u>B. fragilis</u> were screened by Southern hybridization for the location of the resistance determinant. Results indicated that the determinant had inserted into the bacterial chromosome at random sites. Further analysis showed that in 60% of the clones tested, a second copy of the direct repeat sequence had also integrated into the chromosome but in these cases the insertion occurred at a specific site. These results indicate that the Cln^r determinant is located within a classic composite transposon, and provides preliminary evidence that the directly repeated sequences can act as insertion elements in <u>Bacteriodes spp.</u></p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00415-03 LMM																					
PERIOD COVERED October 1, 1985 to September 30, 1986																							
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) The Molecular Biology of Retroviruses Associated with AIDS																							
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: Malcolm A. Martin</td> <td style="width: 33%;">Chief</td> <td style="width: 33%;">LMM, NIAID</td> </tr> <tr> <td>Others: Theodore Theodore</td> <td>Research Microbiologist</td> <td>LMM, NIAID</td> </tr> <tr> <td>Akio Adachi</td> <td>Visiting Fellow</td> <td>LMM, NIAID</td> </tr> <tr> <td>Howard Gendelman</td> <td>Expert</td> <td>LMM, NIAID</td> </tr> <tr> <td>Daryl Daugherty</td> <td>Medical Staff Fellow</td> <td>LMM, NIAID</td> </tr> <tr> <td>Arnold Rabson</td> <td>Medical Staff Fellow</td> <td>LMM, NIAID</td> </tr> <tr> <td>Sundararajan Venkatesan</td> <td>Expert</td> <td>LMM, NIAID</td> </tr> </table>			PI: Malcolm A. Martin	Chief	LMM, NIAID	Others: Theodore Theodore	Research Microbiologist	LMM, NIAID	Akio Adachi	Visiting Fellow	LMM, NIAID	Howard Gendelman	Expert	LMM, NIAID	Daryl Daugherty	Medical Staff Fellow	LMM, NIAID	Arnold Rabson	Medical Staff Fellow	LMM, NIAID	Sundararajan Venkatesan	Expert	LMM, NIAID
PI: Malcolm A. Martin	Chief	LMM, NIAID																					
Others: Theodore Theodore	Research Microbiologist	LMM, NIAID																					
Akio Adachi	Visiting Fellow	LMM, NIAID																					
Howard Gendelman	Expert	LMM, NIAID																					
Daryl Daugherty	Medical Staff Fellow	LMM, NIAID																					
Arnold Rabson	Medical Staff Fellow	LMM, NIAID																					
Sundararajan Venkatesan	Expert	LMM, NIAID																					
COOPERATING UNITS (if any) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">Thomas Folks</td> <td style="width: 33%;">Expert</td> <td style="width: 33%;">LIR, NIAID</td> </tr> </table>			Thomas Folks	Expert	LIR, NIAID																		
Thomas Folks	Expert	LIR, NIAID																					
LAB/BRANCH Laboratory of Molecular Microbiology																							
SECTION Biochemical Virology Section																							
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892																							
TOTAL MAN-YEARS: 6.5	PROFESSIONAL: 3	OTHER: 3.5																					
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews																							
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Using a variety of molecular biology and biochemical virology techniques, we have examined the structure of the acquired immunodeficiency syndrome (AIDS) retrovirus (RV) and characterized viral gene products synthesized in infected cells. During the past year the nucleotide sequences of the envelope (env) genes of an African and North American AIDS viral isolate have been determined. When their deduced amino acid sequences were aligned with the envelopes of the LAV and ARV isolates, conserved and divergent regions were readily identified. Hypervariable stretches of 28 to 74 amino acids, exhibiting 20 to 30% amino acid identity at each position and characterized by reciprocal insertions and deletions, were confined to the gp120 external env protein. The env gene diversity, observed among different AIDS RV isolates, suggests the involvement of the immune system at some point during viral infection and does not augur well for rapid development of an effective vaccine.</p> <p>We have constructed an infectious molecular clone of acquired immunodeficiency syndrome-associated retrovirus. Upon transfection, this clone directed the production of infectious virus particles in a wide variety of cells in addition to human T4 cells. Infectious progeny virions were synthesized in mouse, mink, monkey, and several human non-T cell lines indicating the absence of any intracellular obstacle to viral RNA or protein production or assembly.</p> <p>Thirteen adherent human non-lymphocyte cell lines also were tested for their susceptibility to infection by the AIDS retrovirus. Productive infection could be demonstrated in 3 or 5 colorectal carcinoma cell lines examined; the other 8 human non-lymphocyte cell lines were uninfected. A susceptible colon carcinoma cell line (HT29) as well as normal colonic mucosa were shown to contain a 3.0 kb species of polyadenylated CD4 RNA whereas uninfected colon carcinoma and rhabdomyosarcoma cell lines synthesized no detectable T4 RNA. A persistently infected colon carcinoma cell line was established that continued to produce progeny AIDS virus for more than 10 weeks post-infection.</p>																							

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00433-02 LMM
PERIOD COVERED October 1, 1985 to September 31, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Use of Retroviruses as Tagged Insertional Mutagens		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Jonathan Silver	Medical Officer LMM, NIAID
Others:	M. David Hoggan	Senior Scientist LMM, NIAID
COOPERATING UNITS (if any)		
	Cindy Edwards	Scientist LDP, NCI
LAB/BRANCH Laboratory of Molecular Microbiology		
SECTION Viral Biology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0	0.5
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Proviral insertion into chromosomal DNA is associated with mutation at the site of integration. In systems in which particular mutations can be selected, retroviruses can be used to clone specific cellular genes through the cloning of provirus-cellular DNA junction fragments. With Dr. Cindy Edwards in the Laboratory of Developmental Pharmacology, we are attempting to identify cells in which a Friend murine leukemia provirus has inserted into the gene for the aryl hydroxylase receptor. We plan to use these cells to clone the gene for this receptor. With Dr. M. David Hoggan (LMM), we are screening cells from humans heterozygous for various autosomal recessive cancer genes to see if amphotropic or xenotropic viruses can be used to identify these genes molecularly. Significance: These experiments provide a novel strategy for identifying and cloning genes involved in the metabolism of aryl hydrocarbons and in a variety of human malignancies.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00434-02 LMM
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Studies on the Dissemination of Streptococcal Antibiotic Resistant Determinants		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: Donald J. LeBlanc Head, BVS LMM, NIAID Others: Linda N. Lee Chemist LMM, NIAID		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Molecular Microbiology		
SECTION Bacterial Virulence Section		
INSTITUTE AND LOCATION NIAID, NIH, Frederick, Maryland 21701-1013		
TOTAL MAN-YEARS: <div style="text-align: center;">0.5</div>	PROFESSIONAL: <div style="text-align: center;">0</div>	OTHER: <div style="text-align: center;">0.5</div>
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> A multiply antibiotic resistant strain of <u>Streptococcus faecalis</u>, LDR175, isolated from a broiler in North Carolina, was used as a plasmid donor in conjugation experiments. A 58 kilobase pair (kbp) plasmid, pLDR517, mediating resistance to erythromycin (Em), kanamycin (Km) and streptomycin (Sm), was mobilized into a <u>S. faecalis</u> recipient strain during these experiments. Purified pLDR517 DNA did not hybridize to any of the previously cloned Em, Km or Sm resistance determinants from the well-characterized streptococcal R plasmid, pJH1. The resistance genes from pLDR517 are currently being cloned to determine the extent to which they share homology to DNA from other streptococci with the same resistance phenotype, also previously shown to be unrelated to DNA from pJH1. A unique spectinomycin (Sp) resistance determinant, mediating resistance (2,000 ug/ml) to Sp but not Sm, has been cloned from a plasmid, pDL55, originally from a human clinical isolate of <u>S. faecalis</u>, strain LDR55. The cloned determinant has been sequenced and contains a 783 base open reading frame encoding a protein with a predicted molecular size of 18,943 daltons. The Sp resistance gene shares approximately 48% base sequence homology with a similar gene encoded on a plasmid from <u>Staphylococcus aureus</u>, and the respective gene products share between 40 and 50% amino acid sequence homology. The streptococcal and staphylococcal genes are currently being used as hybridization probes with purified DNA from <u>Neisseria gonorrhoeae</u> isolates from patients in Korea expressing high level resistance to Sp but not Sm. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00437-02 LMM
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) The Biology and Genetics of the AIDS Retrovirus		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	M. David Hoggan	Senior Investigator LMM, NIAID
Others:	Thomas A. Folks	Expert LIR, NIAID
	Howard E. Gendelman	Expert LMM, NIAID
	Scott Koenig	LIR, NIAID
	Malcolm A. Martin	Chief LMM, NIAID
COOPERATING UNITS (if any) <div style="text-align: center;">None</div>		
LAB/BRANCH Laboratory of Molecular Microbiology		
SECTION Viral Biology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	1	1
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unraducad type. Do not exceed the space provided.) <p>Early studies were directed towards a better understanding of the early stages of AIDS retrovirus (RV) infection, such as adsorption as well as the later morphogenesis of new virus particles. These studies were carried out by the electron microscopic examination of the progressive changes produced by acute productive infection with the lymphadenopathy-associated-virus (LAV) strain. During the acute cytolytic infection, many crescent shaped budding particles as well as large numbers of mature particles containing variably shaped condensed nucleoid structures accumulated on the surface of the infected cells. We also examined the chronically infected 8E5 cell line which produces viral RNA and antigens but no infectious virus or reverse transcriptase (RT). Huge numbers of atypical particles were seen at the cell surface. These particles contained various amounts of nucleic acid but few particles containing the condensed nucleoid structures, so prominent during productive infection, could be found.</p> <p>Restriction enzyme analysis of an AIDS retrovirus isolated approximately 30 days after infection of an adult chimpanzee with a pool of five different AIDS isolates, clearly demonstrated the dominance of one of the viruses injected (the Ziare 2 strain) over the other four isolates.</p> <p>Using three different enzymes, no differences could be detected between a pair of isolates from a husband and wife collected the same day. Similarly no gross differences between three isolates from a single AIDS patient could be detected.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00438-02 LMM
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) The Molecular Biology of Cellular Oncogenes		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Eugene M. Santos	Visiting Associate LMM, NIAID
Others:	Theodore Bryan Mercedes Tamame Mariano Barbacid	Microbiologist LMM, NIAID Visiting Fellow LMM, NIAID Section Head DOS, NCI (FCRF)
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Molecular Microbiology		
SECTION Biochemical Virology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 3	PROFESSIONAL: 2	OTHER: 1
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> <u>ras</u> Gene Activation and Carcinogenesis. We have identified activating point mutations in <u>ras</u> genes in about 15% of the most common forms of human cancers. By using RFLP assays we have also demonstrated the somatic nature of these activating point mutations. </p> <p> Amplification of otherwise unaltered <u>ras</u> proto-oncogenes may also lead to malignant transformation. We have demonstrated that the combined effect of multiple copies of the human H-<u>ras</u>-1 proto-oncogene induces malignant transformation of NIH/3T3 cells. We have also reported that amplification of <u>ras</u> genes can be observed in human tumors although at a relatively low frequency. These results show that <u>ras</u> gene amplification occurs in unmanipulated tumor biopsies and, therefore, is not a consequence of <i>in vitro</i> establishment of cell lines. Moreover, they indicate that gene amplification is an alternative pathway by which <u>ras</u> can also contribute to neoplastic development. </p> <p> Monoclonal Antibodies Against <u>ras</u> p21 Proteins. Computer analysis of the predicted amino acid sequences of normal and transforming <u>ras</u> p21 proteins indicates the existence of significant structural differences. These observations raised the possibility that monoclonal antibodies may be elicited against the structural domains specific for transforming p21 proteins. These antibodies could be valuable diagnostic reagents and would be useful to study the functional role of <u>ras</u> p21 proteins. </p> <p> The availability of large amounts of highly purified normal and transforming <u>ras</u> p21 synthesized in <i>E. coli</i> has made possible the development of immunization and screening protocols. A panel of positive hybridomas are being currently screened against purified normal and transforming <u>ras</u> p21 proteins for their ability to exhibit differential affinity for either of the two structural forms of p21, and to interact with the known biochemical activities of these proteins. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00464-01 LMM
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Plasmid Replication and Expression in Streptococci		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Jon M. Ranhand Senior Scientist LMM, NIAID		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Molecular Microbiology		
SECTION Bacterial Virulence Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) <p>Chimeric plasmids were constructed in order to establish if the tetracycline (Tc)-resistance (Tc^r)-determinant that was contained on the <u>Streptococcus faecalis</u> plasmid pAM 1 could be expressed in <u>Streptococcus sanguis</u>. pAMα1 does not transform these cells. The chimeras were constructed from a 5 kb <u>EcoRI</u> fragment that was derived from pAMα1 and a 3.3 kb <u>EcoRI</u> fragment that was derived from pAMα1. The 5 kb fragment contained a replication origin that was known to function in <u>S. sanguis</u> whereas the 3.3 kb fragment contained a Tc^r-determinant and a replication origin that was known to function in <u>Bacillus subtilis</u>. When these chimeras were added to a competent culture of <u>S. sanguis</u> Wicky, a family of Tc^r plasmids, of various sizes, was isolated from them. The replication of members in this family of plasmids, designated pRAN1, pRAN4, pRAN5, pRAN16, and pRAN20, is being investigated. These plasmids are of interest because they contain totally or in part two, independent origins of replication. One of these origins, the one derived from pAMα1, is known to be present in other naturally occurring plasmids that are found in a variety of Gram-positive bacteria. Work in progress is designed to determine which of the two origins of replication is the functional one for each member of the family. In addition, experiments are underway that will provide an understanding as to why pAMα1 does not replicate in <u>S. Sanguis</u>.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00466-01 LMM
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Transcriptional Complexity of AIDS Retrovirus (new)		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Sundararajan Venkatesan	Expert LMM, NIAID
Others:	Nafees Ahmad	Visiting Fellow LMM, NIAID
	Robert J. Mervis	Microbiologist LMM, NIAID
	Arnold B. Rabson	Med. Staff Fellow LMM, NIAID
	Thomas M. Folks	Expert LIR, NIAID
	Malcolm A. Martin	Lab. Chief LMM, NIAID
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Molecular Microbiology		
SECTION Biochemical Virology Section		
INSTITUTE AND LOCATION NIAID, NIH, Frederick, MD 21701		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	1	1
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.) <p> AIDS retrovirus grows to very high titers in a subclass T lymphocytes and the fatal outcome of the disease is thought to be consequent upon selective ablation of a subclass of T lymphocytes. In addition to the usual retroviral <u>gag</u>, <u>pol</u> and <u>env</u> genes, the AIDS RV possesses several additional open reading frames (ORFs) and further augments its coding potential by using complex splicing schemes of mRNA biogenesis. To understand the mechanism of its gene regulation, we have analyzed the viral transcripts by RNA filter blotting and hybridization, nuclease S₁ mapping of the splice junctions and cDNA cloning of viral mRNAs. Three major classes of subgenomic RNAs (5.5, 5.0 and 4.3 kb) were found to possess a 289 base leader spliced to the 3' portion of the genome extending from positions 4542, 4914, 5650, and/or 6100, respectively. A fourth class of mRNA had a variable (1.7-1.9 kb) exon at the 3' end spliced possibly via one or two internal exons to the leader RNA. An additional small splice junction identified near the end of the <u>gag</u> ORF was thought to generate <u>gag-pol</u> fusion mRNA. To determine the functional potential of the different viral transcripts, we have constructed a library of cDNA expression plasmids representing viral RNAs in eukaryotic vectors designed by Okayama and Berg. From this library, we have isolated several unique clones corresponding to differentially spliced class 4 viral mRNAs. By DNA transfection, these plasmids have been introduced into cos cells and the unique viral gene products synthesized in these cells are in the process of being identified by immune detection. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00467-01 LMM
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Structure and Function of Polypeptides Encoded by AIDS RV Genome (new)		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> Sundararajan Venkatesan Others: John E. Coligan Marilyn Lightfoot Robert J. Mervis Thomas M. Folks Malcolm A. Martin </div> <div style="width: 45%;"> Expert Section Chief Staff Fellow Microbiologist Expert Lab. Chief </div> <div style="width: 45%;"> LMM, NIAID LIG, NIAID LIR, NIAID LMM, NIAID LIR, NIAID LMM, NIAID </div> </div>		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Molecular Microbiology		
SECTION Biochemical Virology Section		
INSTITUTE AND LOCATION NIAID, NIH, Frederick, MD 21701		
TOTAL MAN-YEARS: 1.5	PROFESSIONAL: 1.0	OTHER: 0.5
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div style="width: 30%;"> <input type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The genome of the AIDS retrovirus (AIDS RV), the etiologic agent of AIDS codes for several polypeptides in an extremely complex scheme of mRNA biogenesis. Although several of the virus coded polypeptides have been identified and localized on the genetic map by means of partial amino acid sequencing, direct functional correlation of the viral proteins can be greatly facilitated by genetic analysis of viral mutants produced during the natural course of acute infection. Since classical genetic approaches have been hampered by lack of appropriate viral plaquing procedures, we have begun to study viral gene expression in persistently infected cell lines carrying a single copy of integrated viral genome. To facilitate such an approach, we have used cloned cells from a mass of lymphocytes surviving acute infection. One such cell (8E5) was found to contain a single copy of integrated provirus, but produced no viable virus. Lack of reverse transcriptase activity and the absence of two polypeptides of 64 and 34 K in 8E5 cells prompted us to sequence these proteins in wild type virus infected cells. The N - termini of the 64, 51, and the 34 kd polypeptides present an acutely infected cells were localized within the pol reading frame of the proviral DNA. The deduced N-termini of the 64 and the 34 kd proteins were found at 156 and 716 residues from the beginning of the pol open reading frame of ca. 1000 residues. They corresponded respectively to the reverse transcriptase and the endonuclease proteins of the virus. The 51 kd protein had the same N terminus as the 64 kd. Studies are presently in progress to try to determine the exact processing pathway of the gag and the gag-pol precursor proteins. Other viral proteins, notably a 17 kd putative protease and a novel 41 K virus coded proteins are also being sequenced. </p>		

[illegible]

LABORATORY OF PARASITIC DISEASES

1986 ANNUAL REPORT

Table of Contents

SUMMARY	11-1
Project Number	
Z01 AI 00094-27 LPD <u>Entamoeba histolytica</u> : Nutrition, Differenti Virulence, Molecular Probes - Diamond.....	11-13
Z01 AI 00097-28 LPD Physiological and Cytochemical Pathology of Parasitic Diseases - Mercado	11-14
Z01 AI 00098-30 LPD Biochemical Mechanisms of Energy Metabolism in Mammalian and Parasitic Organisms - Weinbach.....	11-15
Z01 AI 00099-16 LPD Biophysical Parasitology - Dvorak.....	11-16
Z01 AI 00102-12 LPD Pathogenesis of Disease Caused by Infection with Intracellular Parasites - Neva.....	11-17
Z01 AI 00103-19 LPD Immunological Studies on Toxoplasmosis and Other Parasitic Diseases - Lunde.....	11-18
Z01 AI 00108-15 LPD Studies on the Biology and Immunogenicity of Malaria Sporozoites - Gwadz.....	11-19
Z01 AI 00161-09 LPD Immunochemistry of Parasitic Diseases - Nash.....	11-20
Z01 AI 00162-10 LPD Biochemical Cytology of Host-parasite Interactions in Parasitic Protozoa - Dwyer.....	11-21
Z01 AI 00197-07 LPD Immunoregulation and Immune Recognition in Filariasis and Non-filarial Diseases - Nutman.....	11-22
Z01 AI 00208-06 LPD The Isolation and Charaterization of Plasmodial Genes - McCutchan.....	11-23
Z01 AI 00240-05 LPD Culture, Physiology and Antigenic Analysis of Sexual Stages of Malaria Parasites - Carter.....	11-24
Z01 AI 00241-05 LPD Identification of Receptors for Merozoite Invasion of Erythrocytes - Miller.....	11-25

Z01 AI 00242-05 LPD	Biological and Biochemical Studies of Antigens on Malaria-infected Red Cells - Howard.....	11-26
Z01 AI 00244-05 LPD	Developmental Adaptations of <u>Trypanosoma cruzi</u> to the Vertebrate Immune System - Sher.....	11-27
Z01 AI 00246-04 LPD	Molecular Studies of the Genome and Surface of <u>Schistosoma mansoni</u> - Sher.....	11-28
Z01 AI 00248-05 LPD	Genetics and Physiology of Vector Capacity in Anopheline Mosquitoes - Gwadz.....	11-29
Z01 AI 00251-05 LPD	Immunologic Studies on Schistosomiasis - Sher.....	11-30
Z01 AI 00253-05 LPD	Studies of the Immunologic Responses to Filarial Infections - Ottesen.....	11-31
Z01 AI 00255-05 LPD	Studies of the Immunologic Responses to Non-Filarial Helminth Infections - Ottesen.....	11-32
Z01 AI 00256-05 LPD	Differentiation of <u>Leishmania</u> Promastigotes - Sacks.....	11-33
Z01 AI 00257-05 LPD	Immunology of Strongyloidiasis - Neva.....	11-34
Z01 AI 00258-04 LPD	Development of Models for Chagas' Disease using <u>T. cruzi</u> Clones and Inbred Mice - Postan.....	11-35
Z01 AI 00347-04 LPD	Schistosomal Hepatic Fibrosis - Cheever.....	11-36
Z01 AI 00348-04 LPD	Immunity in Murine Schistosomiasis - Cheever.....	11-37
Z01 AI 00350-04 LPD	DNA Analysis of Parasites - Nash.....	11-38
Z01 AI 00351-04 LPD	Parasite and Host Factors Controlling the Pathogenesis of Leishmaniasis - Scott.....	11-39
Z01 AI 00439-02 LPD	Clinical and Therapeutic Studies in Human Filariasis - Ottesen.....	11-40
Z01 AI 00483-01 LPD	Plasmodium Chromosome Organization and Genomic Restructuring in Response to Stress - Wellems.....	11-41

Laboratory of Parasitic Diseases

National Institute of Allergy and Infectious Diseases

Summary - October 1, 1985 - September 30, 1986

ADMINISTRATIVE AND ORGANIZATIONAL EVENTS

The new Administration of the Institute has taken over very smoothly, and we look forward to working with Dr. John Gallin, the new Intramural Director and his Administrative Officer, Mary Ann Anerino. The last year has been a latent period regarding the "round robin" move of LPD to Building 4, which has now been emptied for renovation. Budget restrictions and a new system of budget planning which shifts greater responsibility upon individual labs have combined with personnel restrictions (the FTE situation) to keep LPD on a short leash!

The following professional personnel departed for other jobs or other activities: Dr. Rabia Hussain took a faculty position at the Aga Khan Medical School in Pakistan, but will return to LPD as a Guest Worker during summers for 3 years under a Rockefeller Foundation fellowship; Dr. Nirbhay Kumar has also assumed a faculty position at nearby Johns Hopkins Medical School but will continue collaborative work with LPD; Drs. Miriam Postan and Dan Zilberstein returned to Argentina and Israel, respectively, to take academic jobs. Medical Staff Fellow, Vaughn Kirchhoff took a faculty position at University of Iowa and Beth Ungar is temporarily between jobs in the Washington area; two senior Guest Researchers from abroad returned home to their academic positions - Jose R. Coura in Brazil and David Walliker in Scotland; another Guest Worker, Dr. Bart Curry from Australia took a job in Papua, New Guinea. Giovanni Widmer returned to the U.K. to finish his doctorate. We enjoyed the short but intensive visit of Dr. Carlos Ponce and his co-working wife, Elisa, from Honduras. Other LPD departures included Paul Shade, a long-time NIH laboratory assistant, retired and Fran Goodyear, the Chief's secretary took another position with less commuting problems, but we have been fortunate in having an experienced replacement in Linda Tripp.

Incoming professional personnel include the following: Two new Guest Researchers with the Malaria Section are Chiang Syin, Ph.D. and Walter Weiss, M.D., a physician from the Naval Medical Research Institute. Three new Visiting Fellows from Latin America are Juan Carlos Engel, Ph.D. and his wife, Patricia Doyle Engel, Ph.D. both of Argentina who are working with Drs. Dvorak and Weinbach, respectively, and Rosangela da Silva, M.D. of Brazil who is working with Dr. Sacks in Immunology. A man and wife team from India, Alok and Sudha Bhattacharya, both Ph.D.s, spent most of the year working with Dr. Diamond on amebiasis under a special fellowship from the World Bank. The only new Medical Staff Fellows are Anne DeGroot and Peter Melby, both under the auspices of National Research Science Awards Fellowships of the U.S.P.H.S.

As is usual, a number of the LPD staff traveled abroad to attend meetings, workshops, site visits, etc. - often with outside NIH funding; this travel is not detailed here. Travel for purposes of field research was undertaken by Drs. Sacks and Neva in Patna, India for work in kala azar; by Dr. Ottesen in India on filariasis, and by Drs. Nutman, Davey and Ottesen in Guatemala on onchocerciasis.

HONORS AND AWARDS

Many of the LPD staff contribute to the administrative functioning of the NIH, NIAID and even at the level of the Laboratory by serving on Promotion and Tenure Committees, Library Committees, Safety Committee, etc. - as well as a number of Ad Hoc Committees of the Institute organized to address specific issues such as computer use and data processing.

In addition to the above, many of the professional staff serve on WHO Expert Committees and Steering Committees and review research grants for various granting agencies. They also are invited participants to meetings or workshops, and spend a great deal of time reviewing manuscripts. Such recognition and honors are too numerous to be mentioned individually.

The following items, therefore, are considered to merit special mention:

Dr. Sher was recipient of a NIH Director's Award, and continues to serve as co-director of the well-known Biology of Parasitism summer course at Woods Hole.

Dr. Ottesen received an Outstanding Service Medal and is on the Medical Advisory Committee of the Edna McConnell Clark Foundation.

Dr. Neva received a Presidential Meritorious Executive Award and gave the annual Joseph Smadel Lecture of the Infectious Disease Society of America.

Dr. Miller was made a Fellow of the Queensland Institute of Medical Research and gave the invited Derrick-Mackerras Lecture of the University of Queensland in Brisbane, Australia.

Special mention must also be made of the publication of the Bibliography of Chagas' Disease, an indexed compilation of the world literature citations on this subject since 1968. This was organized and initiated primarily by Dr. James Dvorak with the collaboration of C.C. Gibson of the Division of Research Services, and A. Malkelt of Venezuela. The costs of the project were shared by NIAID, PAHO and WHO.

RESEARCH PROGRESS

IMMUNOGENICITY

AND GENE

CLONING OF

MALARIAL ANTIGENS

More basic work needed on recombinant sporozoite vaccines? Synthetic peptides of the circumsporozoite (CS) protein of *P. falciparum*, containing the repeating epitope, NANP, as well as a recombinant antigen produced in bacteria, gave good antibody responses in mice and rabbits (WRAIR collaborators with

Miller). However, when the recombinant (CS) antigen, R32tet32, adsorbed with alum, was tested as an experimental vaccine (FSV-1) in a Phase I trial for safety and immunogenicity in 15 human volunteers, the antibody responses were poor and there was no booster effect. The planned Phase II trial for efficacy has been postponed until or unless better immune responses are obtained (Neva, Sherwood, Miller and WRAIR collaborators). Studies in various in-bred strains of mice immunized with the recombinant CS antigen, as well as with variable length repeating epitopes, suggests that poor helper T-cell recognition partially accounts for the poor immune response to FSV-1 vaccine in humans. Only mice carrying the I-A^b gene responded to the repeating epitope of the CS recombinant antigen. These findings may have broader implications for peptide vaccine development - a requirement of both B and T epitopes in a single amino acid sequence (Good, Miller, Maloy, Berzofsky of NCI and Hockmeyer of WRAIR). In order to work out more precisely the genetic control over immune response to synthetic and recombinant CS antigens, an experimental system that readily permits testing of functional immunity to sporozoite induced malarial infection is needed. This should now be possible since the CS protein of murine malaria (*P. yoelii*) has now been sequenced and synthetic peptides can be prepared to test model vaccines (McCutchan, Lal and de la Cruz).

Circumsporozoite (CS) protein genes of other malarial species.

In addition to the CS protein gene for *P. yoelii* mentioned above, the genes for CS proteins that have been cloned include the other major human malaria, *P. vivax*, and the simian parasite *P. brasilianum* which is similar to or identical with the human *P. malariae*. The immunodominant epitopes on sporozoites of all of these malarial parasites consist of a region of repeating amino acid sequences, flanked by conserved regions I and II on either end of the CS protein molecule (McCutchan, Lal, de la Cruz and numerous collaborators). In addition, CS gene sequences of *P. falciparum* from diverse geographic areas of the world (Asia, East and West Africa and S. America) have been compared and found to vary somewhat, but the immunodominant area of the molecule appears to be stable (McCutchan, Lal, de la Cruz). In contrast, it is interesting that different geographic isolates of a simian malaria, *P. cynomolgi*, exhibit considerable antigenic diversity in their CS proteins. This diversity is located in the middle region of the gene encoding strain-specific repetitive immunodominant epitopes (Gwadz with NYU collaborators).

Cloning and sequencing histidine-rich protein genes. Two genes of *P. falciparum* that produce proteins extraordinarily rich in histidine have been cloned and sequenced. The two proteins have analogous nucleotide sequences, differing only in the sixth amino acid of hexapeptide repeats. One of the proteins, PfHRP II, has been shown by immunoelectronmicroscopy to be transported in "secretory packets" through the parasite as well as the red cell membrane out into the extracellular medium (Wellems, Howard and Atkawa of Case Western). The search for the elusive "cytoadherent protein," responsible for attachment of infected *P. falciparum* red cells to endothelium, continues by screening genomic expression libraries and cDNA libraries of the parasite with Aotus monkey antisera - but still no success (Saul, McCutchan, Howard and Miller).

Chromosomal changes in Plasmodium genome induced by drug pressure and genetic crossing. Mefloquine resistance produced in-vitro in an originally sensitive clone of *P. falciparum* was shown to produce alterations in chromosome size. Drug resistance was retained when drug pressure was removed, also indicating that the parasite genome had been modified. Other environmental influences on the parasite when cultured in-vitro, including drug pressure and presence of antibody, were observed to produce gene deletion, duplication and interchromosomal transposition (Wellems). A genetic cross was done with two separate clones of *P. falciparum*, each possessing distinct markers, by growing each to infective gametocytes, infecting mosquitoes with a sexual stage recombination of the 2 clones and recovering the progeny as asexual blood forms from an infected chimpanzee. The result was found to show recombination between parental genetic markers as well as changes in the size of specific chromosomes. The technique of pulse field gel electrophoresis of DNA was used in these studies (Wellems, Walliker and Carter).

Antigenic analysis of sexual stages of malarial parasites. The search for a single antigen on gametes against which transmission blocking immunity will be more effective continues to be arduous and frustrating. Among a new series of five Mabs developed, four identified previously known gamete surface antigens (45/48 and 25 kDa proteins and the 230 kDa protein) and one new Mab reacted with a 20 kDa protein on gametocytes (Kumar, Carter and Quakyi). Human sera from areas of intense *P. falciparum* transmission in Papua, New Guinea often contain antibodies reactive against gamete surface antigens, and are able to suppress *P. falciparum* infectivity to mosquitoes (Carter and Graves). Non-immune humans were found to contain gamete reactive T-cells in the peripheral blood, and reactive T-cell clones could be developed (Good and Carter).

Molecular basis of vector resistance to malaria. In a line of *Anopheles gambiae* refractory to development of the malaria parasite, the ookinete becomes encapsulated with 24 hours after passing through epithelial cells of the midgut. A protein has been identified by 2 D electrophoresis in the midgut of refractory mosquitoes which is not present in susceptibles. It is hoped that an oligonucleotide probe for this protein can be obtained for gene cloning (Vernick). Melanin and the enzyme, diphenol oxidase, are involved in the encapsulation process of ookinetes in resistant

mosquitoes. The mRNA encoded by genes which control production of the melanin enzymes will be compared in susceptible and resistant mosquitoes (Sakai). A cDNA probe from fat body mRNA has been used to screen a gene library of A. gambiae after a blood meal. The strategy here is to search for promoter and other control elements for genes that are turned on during blood feeding in female mosquitoes (Romans). The prospects for introduction of foreign DNA directly into mosquito eggs have been enhanced by improvements in the injection technique which permit >20% survival (Miller and Sakai).

MALARIA PARASITES

AND THE RED CELL

Interactions of antibody with infected red cells. The studies of last year showing agglutination and inhibition of cytoadherence of P. falciparum

infected red cells by convalescent sera have been extended. These convalescent sera were also shown by immunoelectronmicroscopy using Protein A gold to react only at the knob protrusions of infected red cells, not in between the knobs. Sera from malaria immune West African adults also reacted specifically at the surface of knobs on infected cells. Blockade of cytoadherence to melanoma cells correlated with the sero-reactivity described above, and acute phase serum had no blocking effect against the patients' own infected cells. Yet, the immunoprecipitation patterns of malarial proteins with acute and convalescent sera are nearly identical. These findings underscore the significance of the knob associated surface antigen recognized by convalescent serum in immunity to Falciparum malaria (Howard, Rock, Aikawa at Case Western and Marsh in the Gambia).

What part of the thrombospondin molecule is the receptor for P. falciparum infected red cells? Thrombospondin, the adhesive protein made by mammalian endothelial cells and to which P. falciparum infected red cells bind, can be cleaved by proteolytic enzymes. The fragment containing the heparin-binding domain of thrombospondin does not mediate binding. Current studies are utilizing monoclonal antibodies generated against thrombospondin, some of which block attachment, to map domains for cytoadherence (Sherwood, Roberts, Spitalnik of NIHBL and Taylor of Georgetown).

Merozoite surface proteins involved in parasite invasion.

Some clues have emerged in regard to the mechanism of antigenic variation described last year with P. knowlesi. Probes to the 5' and 3' ends of the gene producing the variant merozoite antigen were found to hybridize to two different chromosomes. Since variant isolates may express merozoite proteins of varying molecular weight, or none at all, it is felt that a point mutation or a gene deletion occurred in parasites that were given to immunized animals. (Hudson, Miller, Welles and Klotz). Further evidence has been obtained that glycoporphins A and B and sialic acid are not the sole ligands for invasion by P. falciparum. A clone of P. falciparum, 7G8, invades neuraminidase treated red cells and, therefore, must get in by a sialic acid independent route. This same clone invades a rare red cell [En(a-)] that lacks glycoporphin A and another red cell type (M_kM_k) that lacks glycoporphins A and B (Miller, Hadley and Klotz). In retrospect, it is amazing that the receptor story for merozoites of

P. vivax and P. knowlesi was so relatively simple in involving only the Duffy blood group antigen! Incidentally, the Duffy antigen binds to antibody on columns and may be purified for further characterization in this way (Miller, Klotz and Hadley).

IMMUNOLOGY OF

LEISHMANIAL

INFECTIONS

Experimental immunization of mice. To overcome technical problems and lack of reproducibility of immunization via the intravenous route, a vaccine model using I.P. injection of soluble antigens with the adjuvant C. parvum was developed.

With a protocol that included one I.P. booster injection of antigen without adjuvant control or healing of lesions in 89 percent of vaccinated mice was demonstrated vs. 100 percent lethal infections in controls. Interestingly, no DTH developed in protected mice, although T-cells were able to produce macrophage activating factor (MAF) and humoral antibody was produced. To identify and separate functional immunogen(s) in the crude antigen, fast protein liquid chromatography (FPLC) was used. Nine fractions or pools obtained by FPLC gave results in immunized mice in which T and B-cell responses were dissociated. Some antigen fractions produced antibody but no T-cell responses, others vice versa and some produced both. Only one pool that gave both T and B-cell responses produced protection. The protective fraction contains several proteins against which monoclonal antibodies have been raised (Scott, Pearce).

Immune response in humans with visceral and cutaneous leishmaniasis and in asymptomatic contacts. Demonstration of the failure of antigen-specific T-cell responsiveness in active or recent cases of kala azar in India was confirmed and extended. In contrast, presumably normal asymptomatic individuals from the same community as active cases of kala azar were found to exhibit both T-cell responses to leishmanial antigen and delayed skin test reactivity. We believe that these immunologically reactive normal people have been infected with L. donovani, but, for reasons yet unknown, were resistant to development of disease (Sacks and Indian collaborators). Leishmanial isolates and studies of T-cell responses continue to be made in patients with cutaneous and mucocutaneous disease referred to us at the NIH Clinical Center. A new DCL case from Mexico was of particular interest because he was specifically anergic, as expected, on entry, but regained T cell responsiveness to leishmanial antigens while on long-term antimony therapy (Neva).

Interaction of leishmanial parasites with complement and macrophages. The "developmental" antigen, which characterizes infective leishmanial promastigotes and can be demonstrated on the cell surface and free in the medium, has very interesting biological properties. This molecule may be responsible for inhibiting the respiratory burst by which macrophages destroy non-infective (log phase) organisms (Sacks). In addition, the uptake and decay of C3 is reduced in infective promastigotes whereas the alternate complement pathway is normally lytic for non-infective organisms. The role of the developmental antigen in promoting complement resistance is under study (Sacks, Joiner and Puentes of LCI).

LEISHMANIAL

Soluble acid phosphatase of *L. donovani*.

BIOCHEMISTRY

This is another substance made by leishmanial parasites that is secreted into the medium, in this case by *L. donovani*.

Polyclonal antibody to this material is cross-reactive with the secreted molecule found to be characteristic of infective stage *L. tropica* by Sacks. However, the acid phosphatase of *L. donovani* is secreted during the log phase of growth and is no longer present on stationary phase organisms. The acid p-tase of *L. donovani* which is 1/3 carbohydrate, with mannose and galactose as primary sugars, has been purified by affinity chromatography for more precise determination of its structure (Bates, Dwyer and Gottlieb of Hopkins).

Sugar transport systems in leishmania. The pentose transport system in *L. donovani* mentioned last year has now been characterized and shown to operate via a facilitated diffusion mechanism (Pastakia and Dwyer). The molecule on the surface membrane of *L. donovani* found to transport glucose has been characterized as a mannosylated glycoprotein of about 19 kDa (Zilberstein and Dwyer).

Identification of sugars in proteins by Western blotting with lectins. The technique of Western blotting with a battery of lectins has been very useful for characterization of various glycoconjugate constituents of leishmania (Dwyer).

BIOLOGY AND

Giardia infections in human volunteers. A

BIOCHEMISTRY OF

clear difference in pathogenicity of two different isolates of *G. lamblia* in human

LUMINAL PROTOZOA

volunteers was demonstrated. With GS/E strain, 10 of 10 became infected and 50 percent were symptomatic, while none of 5 Isr

inoculated volunteers became infected. IgM antibody responses and detection of *Giardia* antigen in stools were found to be more reliable indicators of infection than cyst excretion (Nash and Univ. of Maryland collaborators).

Gene organization and structure of *Giardia*. In the process of analyzing DNA of *giardia* isolates, attention was focused upon the gene producing a major 170 kD antigen which is the target of a cytolytic monoclonal antibody. When the gene for the 170 kD antigen was cloned and used as a probe for presence of the antigen it was found that the gene can be present in the absence of the antigen. One situation in which 170 kD antigen expression is lost even though the gene for its production is present is with in-vitro culture of isolates. The biologic significance of this variability in gene expression is not yet known (Adam, Lal, McCutchan and Nash).

Studies with *E. histolytica*. We may be on the verge of the most significant information to date concerning pathogenic capacity of strains of *E. histolytica*. In the last few years consensus has grown for classifying pathogenic and non-pathogenic strains on the basis of distinctive and stable zymodeme (i.e., iso-enzyme) markers. It has now been shown conclusively that the zymodeme can be altered and switched from pathogenic to non-pathogenic, and vice versa, by manipulation of the bacterial flora with which the ameba is associated in-vitro and by conditions of growth. This has now been accomplished

with clonally derived amebae. Furthermore, even though animal tests for virulence of amebae are not totally clear cut, demonstrable changes in the virulence of amebae in-vivo have accompanied changes in the zymodeme (Mirelman and Diamond).

Improved molecular probes for study of *E. histolytica* have been developed in the form of (1) labelled proteins, separated by 2-D gels, (2) a series of monoclonal antibodies and (3) a library of c-DNA clones. (A. and S. Bhattacharya, Diamond). Two novel NADPH dehydrogenase enzymes of aerobic metabolism of *E. histolytica* were found, one previously unknown and absent in other parasites and mammalian tissues (Weinbach).

MOLECULAR BIOLOGY

The SM-97 vaccine antigen appears to be paramyosin, an invertebrate muscle protein. The 97 kD antigen, isolated and purified from adult *S. mansoni*

OF SCHISTOSOMES

worms, was cloned from a cDNA library of size enriched copies in lambda gt-11. The insert of one clone was recloned in M13, sequenced and found to have a purely alpha helical protein structure. Based upon its amino acid composition, molecular weight, and sub-segmental location in the worm, this molecule appears to be paramyosin, a myofibrillar coiled protein which forms the core for myosin in invertebrate muscle. This has recently been confirmed by using published techniques for purifying paramyosin. The material seems to be a unique immunogen, previously unrecognized in microbial systems (Lanar, Pearce and Sher).

Genetic control of drug resistance. Restriction endonuclease treatment of DNA from hycanthone susceptible and resistant schistosome worms, and subsequent Southern blotting with a ribosomal DNA probe showed presence of a new 3.8 kb fragment in DNA of the resistant strain. Because the test of DNA can be done with material from an individual adult worm, this marker shows promise for investigating the genetic basis of drug susceptibility. Since parasites resistant to hycanthone are also resistant to a more commonly used drug, oxamniquine, such genetic probes could be useful in Brazil, where drug resistance has been documented (Brindley, McCutchan, Sher, Lewis of BRI).

IMMUNOLOGY AND

Immune response to Sm-97 antigen. While molecular cloning and sequencing of the

GENETICS OF

Sm-97 antigen proceeds, further work on immunogenicity of the purified antigen

SCHISTOSOMIASIS

has continued. The antigen was purified by affinity chromatography with monoclonal

antibodies and shown to elicit both delayed hypersensitivity and T-cell lymphokine production in vaccinated mice. Three separate experiments using purified antigen have confirmed its protective effect against challenge infection in mice. Also, schistosome extracts depleted of the Sm-97 antigen fail to induce significant protective immunity. Sera from *S. mansoni* infected humans showed only low levels of antibodies to Sm-97 and cross-reacting antibodies were

found in sera of patients with other helminth infections. T-cell responses to the Sm-97 antigen in patients have not yet been fully evaluated. Neither humoral or T-cell reactivity to Sm-97 were found in mice immunized with irradiated cercariae, indicating that a different antigen is involved in this form of immunization (Pearce, Sher, and other collaborators).

Fully successful therapy vs. schistosomiasis requires immune response in mice. Previous work by others suggested that praziquantel treatment of schistosomiasis lacked effectiveness in adult thymectomized mice. Since praziquantel is now the most widely used anti-schistosome drug for humans, this possible synergy with immune status was investigated further. In μ -suppressed mice, which are B-cell deficient and fail to produce antibody, praziquantel was only 25 percent as effective as in control animals. It was shown that a few days after praziquantel treatment of normal mice infected with S. mansoni that antibodies can be detected binding to the surface of adult worms. Thus, the role of antibodies on a drug-damaged integument may be important in action of the drug (Brindley, Sher).

Further evidence was found for the distinction between the size of granulomas around schistosome eggs in the liver and the degree of hepatic fibrosis associated with their presence. For example, as intensity of infection (i.e., egg numbers) increased, there was no corresponding increase in hepatic fibrosis. These variables also vary markedly among inbred strains of mice, and showed no clear relationship to differences in the major histocompatibility complex (Cheever). An ELISA test for detection of circulating worm antigen (the gut associated proteoglycan or GASP) was developed and found positive in sera from patients with schistosomiasis (Lunde and Nash).

IMMUNE RESPONSE

AND TREATMENT

OF FILARIAL

INFECTIONS

IgE and IgG subclasses. As mentioned in last year's report, filariasis patients with high IgE levels frequently also have high levels of IgG4 antibodies. Considerable effort has been expended upon developing reliable anti-subclass monoclonal antibody reagents that can be put on affinity columns to fractionate sera with high levels of blocking antibody into the four most common IgG subclass components. Then, by using a technique for stripping basophils of IgE, it should be possible to test the various IgG subclasses for blocking activity when basophils are passively sensitized with IgE for histamine release assays when exposed to specific antigen (Hussain and Ottesen). The possibility that specific IgG subclass antibodies might be involved in effecting certain types of immunopathology can now be explored. For example, IgG3 responses have been noted primarily in filariasis patients with lymphatic obstruction (Hussain and Ottesen).

Parasite antigens. Specific filarial antigens that activate T-cells to proliferate have been identified by FPLC chromatography and further purification (Lal, Nutman and Ottesen). Increased sensitivity of an ELISA system for detection of circulating filarial antigen has been developed by use of monoclonal antibodies (Mabs). However, since

the determinant to which most Mabs react is phosphorylcholine (PC), and because most humans have anti-PC antibodies which would interfere with the test for circulating antigen, new Mabs without PC, or other antigens must be explored (Lal, Nutman and Ottesen).

Loa loa infection in Peace Corps volunteers. In cooperation with the Peace Corps a double blind study of diethylcarbamazine (DEC) prophylaxis in about 200 volunteers to West African countries has been in progress. Seroconversion and development of clinical disease has occurred in 10-15 percent of the volunteers taking either weekly DEC or placebo, with the code to be broken for analysis of results soon. The experience with Peace Corps volunteers and other loiasis cases referred to us has permitted better recognition and description of this parasitic infection in newly exposed susceptibles (Nutman, Curry and Miller, and Mulligan of Peace Corps).

Treatment of filariasis. A phase II trial of Ivermectin, a new anthelmintic, has been initiated with collaborators in Madras, India. Circulating microfilariae were rapidly cleared, but by 3 months post-treatment, about 10 percent of original circulating microfilariae had returned in the lowest dose treatment group. However, side effects of treatment in patients with microfilaremia were substantial (Ottesen and Indian collaborators). About 1/3 of patients with tropical pulmonary eosinophilia (TPE) treated conventionally with DEC for 3 weeks were found to have persistent, low-grade alveolitis on the basis of bronchial lavage studies. Further study will be done comparing additional treatment with steroids or more DEC (Ottesen, Nutman, Crystal and Indian collaborators).

IMMUNOREGULATION

AND IMMUNE

RECOGNITION IN

HELMINTH INFECTIONS

T and B-cell clones that stimulate antibody production in-vitro. Work has been extended with a T-cell line, or clone, stimulated by parasite antigen to produce IgE in-vitro. First, a number of T-cell clones, transformed by HTLV-1 virus, have been derived from patients with filariasis

or hyper IgE syndrome and shown to produce factors that regulate IgE production. In addition, B-cell lines have been produced by transforming them with EB virus, and some of these lines produce parasite specific IgE. Although not stable by themselves, it appears that stable lines may be produced by hybridizing them with a human lymphoma cell line. EBV-transformed B cells have also been developed from patients with different filarial infections which produce parasite-specific IgG and IgM antibodies (Nutman and Volkman of LCI).

Timing and extent of antigen recognition in different filarial infections. One measure of the hyper-responsiveness of loiasis patients was the fact that by immunoblotting up to 30 parasite antigens were recognized by their sera. Also, some antigens were recognized early and others late in the course of the infection (Nutman, Hussain and Ottesen). Bronchoalveolar lavage fluids from patients with tropical pulmonary eosinophilia were found to recognize

a distinct subset of filarial antigens different from those recognized by serum of the same patient when analyzed by immuno-blotting (Nutman, Ottesen, Crystal and Indian collaborators).

Are eosinophils from patients with parasites different? The eosinophils generated during early stages of hookworm infection in human volunteers were found to be less dense (?due to a change in granules) than "normal" eosinophils. In addition, these parasite stimulated eosinophils had increased chemotactic and superoxide generating capacity (Maxwell, White and Gallin of LCI). In order to better study effector function of eosinophils during parasitic infection, polyclonal and monoclonal antibodies are being prepared against purified eosinophil granule components. These components include major basic protein (MBP), eosinophil derived neurotoxin (EDN), cationic protein (ECP) and the peroxidase (EPO) (Davey, Nutman and Ottesen).

Antigens from the larvae of *S. stercoralis*. Crude antigens of filariform (infective) stage larvae of *S. stercoralis*, from which ELISA serodiagnostic and skin test antigens were prepared, are being analyzed further by surface and metabolic labelling. At least 16 surface proteins, ranging from 200 to < 6 k in size, could be identified by SDS-PAGE after iodination. One surface protein was lost and another was seen after larvae penetrated rat skin. Many of these antigens, as well as ³⁵S-methionine labelled products could be immunoprecipitated with serum from infected humans and monkeys. We are particularly interested in identifying the antigen(s) responsible for immediate skin test reactivity (Brindley, Gam and Neva).

I. CRUZI AND

CHAGAS' DISEASE

Information derived from analysis of clones of *T. cruzi*. Since a heterogeneous population of parasites certainly exists in vector bugs infected with *T. cruzi*, and since genetic diversity has even been demonstrated in isolates from infected humans, the population dynamics of mixtures of clones becomes important. A mathematical model has been developed which predicts the outcome of parasite growth in media, but the behavior of clone mixtures in triatomid vector bugs is more complex to interpret (Finley and Dvorak). When a tubercidin-resistant clone of *T. cruzi* was developed, the resistant organisms were found unable to take up thymidine (Finley and Cooney of LCI). Pulse field gel electrophoresis of the nuclear DNA of *T. cruzi* is being explored for its possible utility in evaluating parasite clones. Clones of *T. cruzi* derived from isolates in Honduras may provide a useful test of clonal analysis in epidemiology because they come from all parts of a relatively restricted geographic area (Dvorak with C. and E. Ponce of Honduras).

Stage specific antigens of *T. cruzi* involved in complement destruction. For further analysis of complement interaction with *T. cruzi*, as well as changes that occur during differentiation, additional monoclonal antibody (Mab) markers to metacyclic trypomastigotes were produced. One was found to react with a surface 75 k component, and several others with the familiar 90 k surface antigen. However, a 115 k component was also recognized by the 90 k Mab (Sher). Metacyclic tryps, normally resistant to complement lysis, become susceptible after treatment with pronase. An explanation for this was found because in pronase treated parasites the affinity of

Factor B for parasite bound C3b was greatly enhanced. Furthermore, pronase treatment was found to remove the 90-110 k surface glycoprotein complex. Thus, this 90-110 k surface component somehow confers resistance of the parasite to complement lysis. It also appears that a substance called "DAF," or decay accelerating factor, present on mammalian cells, which protects them from attack by the alternate complement pathway, may be involved in the T. cruzi evasion of complement activity (Sher and Joiner of LCI).

Anti-trypanosomal factor (ATF) from Pseudomonas fluorescens. Efforts to improve the purification of ATF and to obtain larger amounts for analysis have continued. Contaminating lipopolysaccharide could be removed by adsorption chromatography and the Ito coil, which utilizes counter current chromatography. Mass spectral analysis disclosed presence of 4 saturated fatty acids (Mercado and Rice of NIADDK, Ferrans and Ito of NHLBI).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00094-27 LPD	
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>Entamoeba histolytica: Nutrition, Differentiation Virulence, Molecular probes</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: L. S. Diamond Section Head LPD, NIAID Others: Alok Bhattacharya Guest Researcher LPD, NIAID Sudha Bhattacharya Guest Researcher LPD, NIAID		
COOPERATING UNITS (if any) Department of Biophysics, Weizmann Institute of Science (D. Mirelman) American Type Culture Collection (P. M. Daggett, T. Nerad)		
LAB/BRANCH <u>Laboratory of Parasitic Diseases</u>		
SECTION <u>Growth and Differentiation Section</u>		
INSTITUTE AND LOCATION <u>NIAID, NIH, Bethesda, Maryland 20892</u>		
TOTAL MAN-YEARS: <u>3.75</u>	PROFESSIONAL: <u>2.0</u>	OTHER: <u>1.75</u>
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>A defined basal medium free of complex biological ingredients has been developed for axenic cultivation of <u>Entamoeba histolytica</u>.</p> <p>Based on their zymodemes (isoenzyme profiles), believed to be a stable and inherent property, isolates of <u>E. histolytica</u> have been divided into two groups, nonpathogenic and pathogenic. To date, only members of the latter have been axenized. Axenization of cloned isolates of amebae displaying nonpathogenic zymodemes from healthy humans is reported. Furthermore, these zymodemes can be altered by manipulating the bacterial flora associated with the amebae in vitro. Axenization of amebae displaying a nonpathogenic profile resulted in a shift to a pathogenic type. Reassociation of the amebae with their bacterial flora resulted in return to the original zymodeme. Reassociation of axenized amebae, isolated from patients with amebic disease and displaying pathogenic zymodemes, with bacterial flora from healthy carriers resulted in shifts from pathogenic to nonpathogenic zymodemes. Reaxenization restored the profiles to their original state. Changes in virulence always accompanied shifts in zymodeme. Amebae displaying a pathogenic zymodeme were virulent, and avirulent when displaying a nonpathogenic zymodeme.</p> <p>Three classes of molecular probes with diagnostic potential were developed for <u>E. histolytica</u>. The first consists of protein profiles of <u>E. histolytica</u> and related <u>Entamoeba</u> made by mapping biosynthetically radio-labeled proteins separated by 2-dimensional gel electrophoresis, the second is a catalog of species-specific monoclonal antibodies, and the third consists of isolate-and-species-specific repeated DNA fragments cloned in plasmid vector pTZ18R.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00097-28 LPD
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Physiological and Cytochemical Pathology of Parasitic Diseases		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: T. I. Mercado Research Physiologist PD, NIAID		
Others: V. J. Ferrans Chief, Ultrastructure Section PB, NHLBI Y. Ito Medical Officer IR, TD, NHLBI H. D. Hochstein Chief, Biological Testing and Reference Standards Branch DCA, BOB K. C. Rice Research Chemist LC, NIADDK		
COOPERATING UNITS (if any) Waters Associates (Millipore), Rockville, MD (M.P. Strickler); Shared Services Dept. (J. Beutler), Frederick Cancer Research Facility, NCI; Dept. of Medicinal Chemistry, Univ. of Illinois, Urbana (K. Rinehart)		
LAB/BRANCH Laboratory of Parasitic Diseases		
SECTION Physiology and Biochemistry		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unrounded type Do not exceed the space provided.) Studies on an anti-trypanosomal factor (ATF) from the bacterial species <u>Pseudomonas fluorescens</u> disclosed that adsorption chromatography with high performance silica columns removed most of the inactive components from the ATF, yielding a fraction which was markedly purer. Counter current chromatography (Ito Coil) aided significantly in the production of larger amounts of the purer substance. Both techniques were useful in removing most of the lipopolysaccharide from the lytic fractions, providing a definite advantage for future <u>in vivo</u> chemotherapeutic studies against <u>Trypanosoma cruzi</u> .		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 AI 00098-30 LPD
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Biochemical Mechanisms of Energy Metabolism in Mammalian and Parasitic Organisms		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: E. C. Weinbach Others: P. Doyle C. E. Claggett S. C. Wieder L. Diamond L. Levenbook	Section Head Visiting Fellow Bio. Lab. Tech. (Chemist) Bio. Lab. Tech. (Chemist) Research Zoologist Research Chemist	LPD, NIAID LPD, NIAID LPD, NIAID LPD, NIAID LPB, NIADDDK
COOPERATING UNITS (if any) Department of Biochemistry, University of Stockholm (B.D. Nelson, T. Hundal) Ciba Pharmaceutical Company (J.L. Costa)		
LAB/BRANCH Laboratory of Parasitic Diseases		
SECTION Physiology and Biochemistry		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205		
TOTAL MAN-YEARS: 4	PROFESSIONAL: 2	OTHER: 2
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Calmodulin (CaM) has been isolated from axenized trophozoites of <i>G. lamblia</i> and purified by gel electrophoresis, gel permeation, and ion exchange chromatography. The parasite CaM has properties characteristic of CaM isolated from other eukaryotes: (a) an apparent molecular weight of 16.7 kd, (b) activation of bovine cyclic AMP phosphodiesterase in a calcium-dependent manner, and (c) sensitivity to known CaM antagonists. Its amino acid composition is similar to mammalian CaM; yet some distinct differences in amino acid content are evident. The membrane-bound Ca^{2+} -dependent ATPase of <i>G. lamblia</i> has been characterized, and found to be sensitive to inhibitors of CaM. Two novel enzymes of aerobic metabolism of <i>Entamoeba histolytica</i> were found in studies initiated this year: (1) a Ca^{2+} -activated NADPH dehydrogenase (previously reported only in plants), and (2) a highly active Mn^{2+} -dependent ($K_m = 5 \mu\text{M}$) pyridine nucleotide dehydrogenase specific for NADPH, Mn^{2+} and quinone electron acceptors. Biochemical and pharmacological studies were initiated on <i>Trypanosoma cruzi</i> with an examination of the effects of tricyclic antidepressant drugs on the <i>in vitro</i> growth of the parasite. Similar to our previous findings with the enteric protozoa, chlorimipramine was more toxic to the trypanosomes than was the parent compound imipramine (10 vs. 50 μM). Morphological changes were clearly evident in the drug-treated parasites. Studies of mammalian bioenergetics centered on the effects of diflusalin, a difluorophenyl derivative of salicylic acid, on oxidative phosphorylation. The drug has powerful uncoupling capacity, releasing respiratory control and impeding synthesis of ATP in rat liver mitochondria at low concentrations ($\text{IC}_{50} = 20 \mu\text{M}$). This is another example where substitution by a halogen atom (fluorine) increases the pharmacological potency of the parent compound (salicylic acid itself is virtually devoid of uncoupling properties).		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 AI 00099-16 LPD															
PERIOD COVERED October 1, 1985 to September 30, 1986																	
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Biophysical parasitology																	
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation) PI: J. A. Dvorak Res. Microbiologist LPD, NIAID																	
Others: <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">J. C. Engle</td> <td style="width: 33%;">Fogarty Fellow</td> <td style="width: 33%;">INDIECH, Argentina</td> </tr> <tr> <td>R. W. Finley</td> <td>Med. Staff Fellow</td> <td>LPD, NIAID</td> </tr> <tr> <td>C. C. Gibson</td> <td>Engineer</td> <td>BEIB, DRS</td> </tr> <tr> <td>J. Kwon-Chung</td> <td>Res. Microbiologist</td> <td>LCI, NIAID</td> </tr> <tr> <td>M. Postan</td> <td>Visiting Associate</td> <td>LPD, NIAID</td> </tr> </table>			J. C. Engle	Fogarty Fellow	INDIECH, Argentina	R. W. Finley	Med. Staff Fellow	LPD, NIAID	C. C. Gibson	Engineer	BEIB, DRS	J. Kwon-Chung	Res. Microbiologist	LCI, NIAID	M. Postan	Visiting Associate	LPD, NIAID
J. C. Engle	Fogarty Fellow	INDIECH, Argentina															
R. W. Finley	Med. Staff Fellow	LPD, NIAID															
C. C. Gibson	Engineer	BEIB, DRS															
J. Kwon-Chung	Res. Microbiologist	LCI, NIAID															
M. Postan	Visiting Associate	LPD, NIAID															
COOPERATING UNITS (if any) None																	
LAB/BRANCH Laboratory of Parasitic Diseases																	
SECTION Physiology and Biochemistry Section																	
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892																	
TOTAL MAN-YEARS: 2.0	PROFESSIONAL: 1.0	OTHER: 1.0															
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews																	
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided) <p> This project is concerned with studies of the genetic diversity of <u>Trypanosoma cruzi</u> and its implications to the epidemiology and diagnosis of Chagas' disease. Emphasis during the year has centered upon: 1) Completion of the analysis of the infection of inbred mice with <u>T. cruzi</u> clones, 2) Analysis and modeling of the population dynamics of <u>T. cruzi</u> clone mixtures, 3) Development of mutant <u>T. cruzi</u> clones, 4) Pulse gel electrophoretic analyses of <u>T. cruzi</u> clones, 5) Utilization of PARACYT-1, the LPD flow cytometer, to study the DNA synthetic cycle of <u>Giardia lamblia</u> and <u>Candida</u> spp. and 6) Completion of a Medlars-based bibliography on Chagas' disease. </p>																	

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00102-12 LPD
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Pathogenesis of Disease Caused by Infection with Intracellular Parasites		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: F. A. Neva Chief LPD, NIAID		
Others: D. Sacks Senior Staff Fellow LPD, NIAID P. A. Scott Senior Staff Fellow LPD, NIAID C. Lane Senior Investigator LIR, NIAID J. A. Sherwood Medical Staff Fellow LPD, NIAID		
COOPERATING UNITS (if any) Univ. of Arizona, Tucson (E.A. Petersen); Harvard Med. School, Boston, MA (F. von Lichtenberg); Inst. of Dermatology, Santa Domingo, Dominican Republic (H. Bogaert); Instituto de Salubridad y Enfermadades Tropicales, Mexico City (O. Velasco); Rajendra Memorial Inst., Patna, India (L. Prasad and staff).		
LAB/BRANCH Laboratory of Parasitic Diseases		
SECTION Cell Biology and Immunology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205		
TOTAL MAN-YEARS: 1.6	PROFESSIONAL: 1.1	OTHER: 0.5
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The focus of this project continues to be on properties of leishmanial parasites isolated from patients and the immune response of the infected human host. Conventional parasitological techniques are used, and immune response of the host is assessed by cell-mediated immune responses such as lymphocyte proliferation, generation of lymphokines (IL-2 and γ -interferon) and delayed hypersensitivity skin test. Parasite isolates from cutaneous cases originating in Surinam were obtained, as well as isolates from visceral infections in India. Several of the latter isolates were from patients who failed to respond to Pentostam and Pentamidine therapy. A new patient with diffuse cutaneous leishmaniasis (DCL) from Mexico was studied. He apparently recovered cellular immune responsiveness while receiving intensive antimony therapy. Pathogenicity of leishmanial isolates, as well as the sequence in pathologic events during infection in the ear of BALB/c mice, is also being studied. A phase I trial of safety and immunogenicity of a <i>P. falciparum</i> anti-sporozoite vaccine is in progress in collaboration with the Walter Reed Army Institute of Research (WRAIR). The vaccine is a recombinant protein (R32 tet 32), produced in <i>E. coli</i> and absorbed with alum. Fifteen human volunteers have received 10 to 800 μ g doses at monthly intervals. One recipient had an allergic reaction after the third dose. Humeral antibody responses were low with no booster effect and cell-mediated responses are still being evaluated.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00103-19 LPD
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Immunological Studies on Toxoplasmosis and Other Parasitic Diseases		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: M. N. Lunde Research Zoologist LPD, NIAID		
Others: A. W. Cheever Assistant Chief LPD, NIAID L. Jacobs Scientist Emeritus NIAID T. E. Nash Senior Investigator LPD, NIAID E. A. Ottesen Senior Investigator LPD, NIAID		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Parasitic Diseases		
SECTION Host-Parasite Relations Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205		
TOTAL MAN-YEARS: 1.5	PROFESSIONAL: 1.1	OTHER: 0.4
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The presence of <u>Toxoplasma</u> endozoites in tissue from chronically infected mice was demonstrated by differences in survival of endozoites and cysts in pepsin-HCl. This may be important in explaining reactivated toxoplasmosis in immuno-compromised hosts. An ELISA for the detection of gut-associated circulating anodic proteoglycan (GASCAP) using specifically labeled antibody to GASCAP has been developed. Using this assay, circulating antigen has been found in sera from patients with schistosomiasis. Antigen, however, was not detected in sera from patients two months post-treatment.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00108-15 LPD
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Studies on the Biology and Immunogenicity of Malaria Sporozoites		
PRINCIPAL INVESTIGATOR (List other professional persons below the Principal Investigator.) (Name, title, address and institute affiliation) <div style="display: flex; justify-content: space-between;"> <div style="width: 30%;"> Others: T. McCutchan W. Weiss A. Lal M. L. Maloy I. Quakyi T. Quinn S. Francis </div> <div style="width: 30%;"> Guest Worker Senior Scientist Guest Worker Senior Scientist Guest Worker Senior Scientist Head, Malaria Section </div> <div style="width: 30%;"> LPD, NIAID LPD, NIAID LPD, NIAID LPD, NIAID LIC, NIAID LPD, NIAID LIR, NIAID LPD, NIAID </div> </div>		
COOPERATING UNITS (if any) WRAIR (W. Hockmeyer); NAMRI, L. H. Miller, J. A. Berzofsky, Senior Scientist, Metabolism Branch, NCI.		
LAB/BRANCH Laboratory of Parasitic Diseases		
SECTION Malaria		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 1.6	PROFESSIONAL: 1.2	OTHER: 0.4
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.) <p> Circumsporozoite (CS) proteins have been shown to act as the <u>protective antigens</u> capable of inducing <u>sporozoite</u> - <u>specific</u> immunity in several species of malaria. The gene coding for this antigen in <u>Plasmodium falciparum</u> has been isolated, cloned and incorporated into <u>E. coli</u>. The recombinant protein induces antibodies in mice which react with sporozoites and blocks liver invasion <u>in vitro</u>. </p> <p> T cells are important for the development of protective immunity to malarial sporozoites. The repeating epitope, NANP, of <u>P. falciparum</u> circumsporozoite (CS) protein is recognized by T cells from mice which carry the I-A^B gene. Evidence suggests that the human immune response to this epitope may be very restricted. This has serious implications for vaccine development. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00161-09 LPD
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Immunochemistry of Parasitic Diseases		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: T. E. Nash Others: F. A. Neva R. A. Adam		
COOPERATING UNITS (if any) M.M. Levine, D.A. Herrington, G.A. Lysonsky, University of Maryland		
LAB/BRANCH Laboratory of Parasitic Diseases		
SECTION		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205		
TOTAL MAN-YEARS: 3.3	PROFESSIONAL: 1.8	OTHER: 1.5
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The pathogenicity of <i>Giardia lamblia</i> for man was definitively established. 10/10 individuals inoculated with GS/E became infected and 50% became ill. Differences in the pathogenicity of isolates was additionally demonstrated because none of the 5 Isr inoculated volunteers became infected. IgM responses appear to be the most reliable humoral indicator of infection since all infected persons showed significant IgM antibody responses. <i>Giardia</i> antigens were reliably detected in the stools of infected volunteers despite the variation in cysts excretion. Analysis of the surgace antigens and E-S products of new <i>Giardia</i> isolates confirms the diverse heterogeniety of <i>Giardia lamblia</i> in nature. The ability of <i>Giardia</i> to persist in culture despite the presence of high concentrations of antibiotics was studied. Persistence was dependent on the concentration of antibiotics, duration of exposure, inoculum size and stability of the antibiotic.</p> <p>Ultrasonography reliably detects Symmers' fibrosis. This technique is now being applied in the study of infected populations in the field. An assay to detect circulating GASP antigen in humans was established.</p> <p>Antibody responses to specific <i>Cryptosporidium</i> antigens was studied using Western blots. Most infected persons recognized a 23kd antigen.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00162-10 LPD
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Biochemical Cytology of Host-Parasite Interactions in Parasitic Protozoa		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: D. M. Dwyer Supervisory Microbiologist LPD, NIAID Others: P. A. Bates Visiting Fellow, FIC LPD, NIAID K. A. Joiner Senior Investigator LCI, NIAID M. O. Odera Guest Investigator LPD, NIAID K. B. Pastakia Senior Staff Fellow LPD, NIAID S. M. Puentes Clinical Fellow LCI, NIAID D. Zilberstein Visiting Fellow, FIC LPD, NIAID		
COOPERATING UNITS (if any) Dept. of Immunol. & Infect. Dis., Johns Hopkins Univ. (M. Gottlieb); Naval Bio- sciences Lab., Sch. Pub. Hlth., Univ. California, Berkeley (H. W. Sheppard); WRAMC (K. D. Burman); Dept. Biochem., Univ. Victoria (R. W. Olafson).		
LAB/BRANCH Laboratory of Parasitic Diseases		
SECTION Cell Biology & Immunology		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205		
TOTAL MAN-YEARS: 4.5	PROFESSIONAL: 4.5	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The cell biology and biochemistry of <u>Leishmania</u> and <u>Trypanosoma</u> are investigated as models of intra- and extracellular parasitism, respectively. As all interactions between host and parasite occur at the level of the parasite surface membrane (SM), emphasis is placed on: 1) its integrated biochemical characterization and 2) defining its roles in parasite survival.</p> <p>A ~ 65 kDa avidin-binding glycoprotein of was identified in the SM of <u>L. donovani</u> (<u>L. d.</u>) promastigotes which could function in carboxylase reactions. The major carbohydrate constituents of both SM and released glycoproteins of <u>L. d.</u> were identified using Western blots. The kinetics of synthesis and secretion of soluble acid phosphatase (SACP) by <u>L. d.</u> were determined and its glycosylation events identified. SACP was purified and its N-terminal amino acid sequence determined. Complement component C₃ binds to the SM of <u>L. d.</u> predominantly as iC₃, and most is released from cells as cleavage fragments presumably resulting from parasite SM protease activity. A facilitated diffusion-mediated pentose transport system was characterized in <u>L. d.</u> The <u>L. d.</u> SM 3'-nucleotidase (3'-NT) was isolated and characterized as a 43 kDa mannose-glycoprotein. Clones putatively encoding for 3'-NT and a SM gp-63 antigen were isolated from a <u>L. d.</u> genomic library. The <u>L. d.</u> SM 5'-nucleotidase was partially purified and putatively identified as a 72 kDa mannosylated glycoprotein. Two distinct proton-ATPases were characterized in the SM and mitochondrion of <u>L. d.</u>, respectively. The <u>L. d.</u> SM D-glucose transporter was characterized as a 19 kDa mannosylated-glycoprotein. Further, a 55-62 kDa thyrotropin binding protein was demonstrated in the SM of <u>L. d.</u></p> <p>The current results provide further functional characterization of parasite SM constituents which might prove useful as targets for chemotherapy and/or as agents for immunoprophylaxis.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00197-07 LPD
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Immunoregulation and Immune Recognition in Filariasis and Non-filarial Diseases		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: T. B. Nutman Senior Staff Fellow LPD, NIAID Others: E. A. Ottesen Senior Investigator LPD, NIAID D. J. Ward Medical Staff Fellow LPD, NIAID R. B. Lal Visiting Scientist LPD, NIAID R. Hussain Senior Staff Fellow LPD, NIAID H. Francis Senior Investigator LIR, NIAID		
COOPERATING UNITS (if any) PB, NHLBI, NIH, Bethesda, MD (R.G. Crystal); Dept. Allergy & Immunol., SUNY, Stony Brook, NY (D.J. Volkmann); Dept. Clin. Immunol., Univ. of Winnipeg, Manitoba, Canada (G. Delespesse); Dept. Medicine, Royal Hammersmith Hosp., London, UK (A.P. Weetman); Dept. Allergy & Clin. Immunol., UCLA, Los Angeles, CA (A. Saxon)		
LAB/BRANCH Laboratory of Parasitic Diseases		
SECTION Host Parasite Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205		
TOTAL MAN-YEARS 1.1	PROFESSIONAL: 0.5	OTHER: 0.6
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The purpose of this project is to delineate the mechanisms involved in regulating the humoral and cellular responses in patients with filariasis and other disease states and to determine those antigens responsible for inducing these responses.</p> <p><u>In vitro</u> models of parasite-antigen driven antibody production as well as parasite-specific human T cell and B cell clones have been developed to understand in more detail those mechanisms regulating antibody production (particularly IgG and IgE) in filariasis, allergic diseases and in the normal situation. Further, HTLV-1 transformed T cell clones bearing T cell activation antigens and Fc epsilon receptors have been made in order to isolate factors involved in the T cell regulation of this isotype (IgE) which appears to be up-regulated in parasitic diseases. These T cells as well as a B cell line bearing the Fc epsilon receptor (FcER) have been used to isolate, purify, and raise antibody to the FcER which has recently enabled the gene for this receptor to be cloned molecularly.</p> <p>Qualitative analysis of the antigens recognized by the immune system at both a B and T cell level has been performed and T cell lines recognizing an <u>Onchocerca volvulus</u> species specific antigen has been constructed. Antigens recognized by human monoclonal antibodies derived from EBV-transformed patient B cells have also been identified. FPLC analysis of the antigenic components of filarial parasites has identified and helped to partially purify certain molecules which appear to regulate the immune response at both the cellular and humoral level human filariasis.</p> <p>Immunoblot analysis of filaria-specific IgE and IgG in loiasis, lymphatic filariasis, and onchocerciasis have indicated patterns of antigen recognition which differ among groups of patients with different clinical manifestations of filariasis and among those with similar manifestations but who have been exposed to the parasite for different lengths of time. 11-22</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00208-06 LPD
PERIOD COVERED <u>October 1, 1985 to September 30, 1986</u>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>The Isolation and Characterization of Plasmodial Genes</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: T. F. McCutchan Others: A. Lal V. de la Cruz J. Welsh W. Weiss M. Good		
COOPERATING UNITS (if any) None		
LAB/BRANCH <u>LPD</u>		
SECTION <u>Malaria</u>		
INSTITUTE AND LOCATION <u>NIAID, NIH, Bethesda, Maryland 20892</u>		
TOTAL MAN-YEARS: <u>4.0</u>	PROFESSIONAL: <u>3.0</u>	OTHER: <u>1.0</u>
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> This laboratory has been involved in contributing to the development of a malarial vaccine. To this end we have been cloning and characterizing surface antigen genes from the human malarias and have been developing a rodent model system for vaccine testing. Two important contributions with regard to human malarias have been the identification of the immunodominant epitopes of antigens that are dis-played on the surface of the Plasmodial sporozoites of <i>P. vivax</i> and <i>P. malariae</i>. Further, an antigen corresponding to the sporozoite antigens of the human malarias has been identified in a rodent malaria. With the goal of developing a rodent model for malaria vaccines, we have immunized mice against this antigen. We are presently challenging the immunized mice with malarial parasites and will characterize the degree of protection afforded by the vaccination as well as the B-cell and T-cell responses. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00240-05 LPD
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Culture, Physiology and Antigenic Analysis of Sexual Stages of Malaria Parasites		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: R. Carter Visiting Scientist LPD, NIAID Others: N. Kumar Burroughs Wellcome Senior Research Fellow LPD, NIAID W.H.O. Fellow LPD, NIAID Hamilton Fairley Fellow LPD, NIAID W.H.O. Fellow LPD, NIAID Staff Fellow LPD, NIAID Visiting Scientist LPD, NIAID		
COOPERATING UNITS (if any) U. of Colombo, Sri Lanka (K. Mendis); Harvard Sch. of Pub. Hlth, Boston (D. Wirth); Papua New Guinea Inst. Med. Res. (P. Graves); Naval Med. Res. Inst., Bethesda, MD (A. Szarfman); Hazelton Laboratories, VA (J. Renner); U. of Edinburgh, Scotland (D. Wallikes).		
LAB/BRANCH		
SECTION Malaria Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 5.7	PROFESSIONAL: 4.2	OTHER: 1.5
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The object of this project is to define and isolate genes coding <u>P. falciparum</u> antigens which are the targets of antibodies which block infectivity of gametocytes of this parasite to mosquitoes. The repertoire of monoclonal antibodies specific to sexual stages of <u>P. falciparum</u> has been expanded to further define target antigens which are shown to include the 230 kDa gamete surface protein of <u>P. faciparum</u>. Cloning genes coding for such antigens has been beset with problems including the immunoselection of DNA clones coding a 70 kDa heat shock protein which appears to be structurally related to the 230 kDa gamete surface protein which was the object of the search.</p> <p>Field studies have shown that human populations exposed to <u>P. falciparum</u> transmission in Papua New Guinea, elaborate antibodies against the 230 kDa and the 48 kDa and 45 kDa gamete surface proteins. Studies with <u>P. vivax</u> in Sri Lanka have shown that certain antigamete antibodies which suppress infectivity of the parasite to mosquitoes at high concentrations, enhance infectivity at low concentrations. Human T-cell clones with specificity for <u>P. falciparum</u> gamete antigens have been isolated from the blood of non-immune individuals. These clones have "helper" T-cell phenotypes and their reactivity is histocompatibility antigen restricted. It is planned to use such clones to define the T-cell epitopes of gamete antigens.</p> <p>Analysis of the cross hybridization experiment between clones of <u>P. falciparum</u> is being continued in several laboratories. Cross hybridization has been shown to lead to changes in chromosome size.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00241-05 LPD
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Identification of Receptors for Merozoite Invasion of Erythrocytes		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	L. H. Miller,	Section Head LPD, NIAID
Others:	F. Klotz	Staff Fellow LPD, NIAID
	R. Howard	Expert Consultant LPD, NIAID
	D. Hudson	Biologist LPD, NIAID
COOPERATING UNITS (if any) WRAIR, Washington, DC (T. Hadley); Hazelton Laboratories, Vienna, VA (J. Renner); Case Western Reserve University, Cleveland, OH (M. Aikawa); Guys Hospital, London (G. Mitchell).		
LAB/BRANCH Laboratory of Parasitic Diseases		
SECTION Malaria Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.) <p>The <u>merozoite</u> interacts in a <u>receptor</u> specific manner with the <u>erythrocyte</u> surface and is the stage against which immunity may work to block <u>invasion</u>. Thus, merozoite surface components are of interest for their role in erythrocyte recognition and as <u>antigens</u> for induction of <u>protective immunity</u>. We are now studying one antigen on the merozoite surface that undergoes antigenic variation to understand the molecular basis for this variation.</p> <p>We are studying <u>P. falciparum</u> mutants that use an alternate receptor for invasion of human red cells and continuing the study of the Duffy blood group molecule that is the receptor for <u>P. vivax</u> invasion.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00242-05 LPD
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Biological and Biochemical Studies of Antigens on Malaria-Infected Red Cells		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: R. Howard Visiting Scientist LPD, NIAID Others: L. H. Miller Section Head LPD, NIAID J. A. Sherwood Clinical Associate LPD, NIAID A. J. Saul Visiting Associate LPD, NIAID L. J. Pantone Guest Worker LPD, NIAID T. E. Wellems Clinical Associate LPD, NIAID E. P. Rock HHMI-NIH Fellow LPD, NIAID W. Daniel Research Associate LPD, NIAID		
COOPERATING UNITS (if any) Case Western Reserve Univ. (M. Aikawa, S. Uri); Immunology Dept. WRAIR (J. Lyon); Med. Res. Council Labs, The Gambia (K. Marsh); LSB, NIADDK (D. D. Roberts, S. J. Spitalnik, V. Ginsburg); LC, NIAMD (K. Kirk, L. Cohen); Georgetown Univ. (D. Taylor); Christian Albrecht's Univ, W. Germany (R. Schauer).		
LAB/BRANCH Laboratory of Parasitic Diseases		
SECTION Malaria Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS 6.5	PROFESSIONAL: 6.25	OTHER: 0.25
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>We are studying the structure and function of malarial proteins inserted into and through the membrane of erythrocytes infected with mature asexual malaria parasites and molecules on the endothelial cells involved in cytoadherence. Two very large ($M_r \sim 300,000$) <u>P. falciparum</u> proteins have been localized to the surface membrane of infected erythrocytes. One protein is exposed on the cell surface and appears to mediate attachment to endothelial cells. The other malarial protein is localized under the membrane at the knob protrusions which mediate cytoadherence. These proteins do not appear to be related structurally. A third <u>P. falciparum</u> protein of unusually high histidine content has also been localized to the submembrane material under knobs. The histidine-rich protein is a structural or functional component of knobs but does not directly confer cytoadherence.</p> <p>Immunoelectronmicroscopy studies of <u>P. falciparum</u> isolates from West African patients have shown that the isolate-specific and pan-specific cell surface epitopes defined by homologous and heterologous African sera are both localized on the knob protrusions.</p> <p>The potential role of thrombospondin as the host cell ligand for cytoadherence has been studied in more detail. The stage-specificity of attachment of infected cells to thrombospondin and trypsin-sensitivity of the infected cell surface component involved in attachment to this protein match the properties of attachment to endothelial cells. We have also shown that the domain of thrombospondin involved in heparin binding is not involved in attachment of infected erythrocytes.</p> <p>We have also sequenced two <u>P. falciparum</u> genes encoding proteins of extraordinary histidine content, one of which is exported <u>through</u> the host erythrocyte surface membrane into the plasma.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00244-05 LPD
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Developmental Adaptation of <u>Trypanosoma cruzi</u> to the Vertebrate Immune System		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: A. Sher	Section Head	LPD, NIAID
Others: K. Joiner	Senior Investigator	LCI, NIAID
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Parasitic Diseases		
SECTION Immunology and Cell Biology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 1.5	PROFESSIONAL: 1.0	OTHER: 0.5
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>In this project we have been studying developmental adaptations of <u>T. cruzi</u> to the vertebrate host, and in particular, surface membrane changes occurring during the morphogenesis of epimastigotes (vector stage) to metacyclic trypomastigotes (infective stage).</p> <p>A. <u>Stage specific antigens recognized by monoclonal antibodies.</u> A series of stage specific monoclonal antibodies were generated against metacyclic trypomastigotes. These antibodies were characterized as recognizing surface antigens with relative M_s of 90 and 75k. The 90k species was found to be partially released into the medium upon culture of the parasites.</p> <p>B. <u>Mechanism of ACP regulation by T. cruzi.</u> As previously described by us, metacyclic trypomastigotes which normally evade lysis by the alternative complement pathway (ACP) can be made sensitive by prior treatment with pronase. We have now shown that this treatment effects ACP activation by facilitating the binding of Factor B to parasite bound C3b, a process which is inefficient in the non-enzyme exposed parasites. In addition, we have shown that the enzyme treatment selectively removes a 90k-115k glycoprotein (recognized by one of the monoclonals mentioned above) from the metacyclic surface, suggesting that this parasite molecule might be responsible for the regulation of ACP activation. Finally, a decay accelerating factor-like activity was detected in the supernatant of cultured metacyclic forms which also could account for the evasion of complement lysis by these vertebrate-adapted parasites.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00248-05 LPD																																
PERIOD COVERED October 1, 1985 to September 30, 1986																																		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Genetics & Physiology of Vector Capacity in Anopheline Mosquitoes																																		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 40%;">R. W. Gwadz</td> <td style="width: 40%;">Senior Scientist</td> <td style="width: 10%;">LPD, NIAID</td> </tr> <tr> <td></td> <td>L. H. Miller</td> <td>Section Head</td> <td></td> </tr> <tr> <td></td> <td>R. K. Sakai</td> <td>Expert</td> <td></td> </tr> <tr> <td>Others:</td> <td>P. A. J. Romans</td> <td>Guest Worker</td> <td></td> </tr> <tr> <td></td> <td>K. D. Vernick</td> <td>Bio. Lab. Tech.</td> <td></td> </tr> <tr> <td></td> <td>R. Carter</td> <td>Visiting Scientist</td> <td></td> </tr> <tr> <td></td> <td>H. G. Coon</td> <td>Senior Investigator</td> <td>LG, NCI</td> </tr> <tr> <td></td> <td>P. Kantoff</td> <td>Senior Investigator</td> <td>LMH, NHLBI</td> </tr> </table>			PI:	R. W. Gwadz	Senior Scientist	LPD, NIAID		L. H. Miller	Section Head			R. K. Sakai	Expert		Others:	P. A. J. Romans	Guest Worker			K. D. Vernick	Bio. Lab. Tech.			R. Carter	Visiting Scientist			H. G. Coon	Senior Investigator	LG, NCI		P. Kantoff	Senior Investigator	LMH, NHLBI
PI:	R. W. Gwadz	Senior Scientist	LPD, NIAID																															
	L. H. Miller	Section Head																																
	R. K. Sakai	Expert																																
Others:	P. A. J. Romans	Guest Worker																																
	K. D. Vernick	Bio. Lab. Tech.																																
	R. Carter	Visiting Scientist																																
	H. G. Coon	Senior Investigator	LG, NCI																															
	P. Kantoff	Senior Investigator	LMH, NHLBI																															
COOPERATING UNITS (if any) Dept. of Entomology, U. of Maryland, College Park, MD (Dr. M. Ma)																																		
LAB/BRANCH Laboratory of Parasitic Diseases																																		
SECTION Malaria Section																																		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205																																		
TOTAL MAN-YEARS: 5.7	PROFESSIONAL: 3.2	OTHER: 2.5																																
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td><input type="checkbox"/> (a) Human subjects</td> <td><input type="checkbox"/> (b) Human tissues</td> <td><input checked="" type="checkbox"/> (c) Neither</td> </tr> <tr> <td><input type="checkbox"/> (a1) Minors</td> <td></td> <td></td> </tr> <tr> <td><input type="checkbox"/> (a2) Interviews</td> <td></td> <td></td> </tr> </table>			<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither	<input type="checkbox"/> (a1) Minors			<input type="checkbox"/> (a2) Interviews																									
<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither																																
<input type="checkbox"/> (a1) Minors																																		
<input type="checkbox"/> (a2) Interviews																																		
SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.) <p>The <u>molecular biology</u> of <u>anopheline mosquitoes</u> is being studied in relation to the capacity of these vectors to transmit <u>malaria</u>. Systems for <u>cloning</u> and <u>transposing genes</u> into <u>mosquito germ lines</u> are being developed. Factors which render mosquitoes refractory to malarial infection, with special emphasis on mechanisms which encapsulate developing parasites, are being defined. Linkage of refractory genes to genes regulating blood-meal associated physiological events, e.g., vitellogenin synthesis, will be used to enhance refractory responses.</p> <p>The ability to identify, clone and transpose genes which regulate refractory mechanisms should permit the development of mosquito lines incapable of transmitting malaria for use in malaria control schemes.</p>																																		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00251-05 LPD									
PERIOD COVERED October 1, 1985 to September 30, 1986											
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Immunologic Studies on Schistosomiasis											
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: A. Sher</td> <td style="width: 33%;">Section Head</td> <td style="width: 33%;">LPD, NIAID</td> </tr> <tr> <td>Others: E. J. Pearce</td> <td>Guest Worker</td> <td>LPD, NIAID</td> </tr> <tr> <td>P. Brindley</td> <td>Fogarty Fellow</td> <td>LPD, NIAID</td> </tr> </table>			PI: A. Sher	Section Head	LPD, NIAID	Others: E. J. Pearce	Guest Worker	LPD, NIAID	P. Brindley	Fogarty Fellow	LPD, NIAID
PI: A. Sher	Section Head	LPD, NIAID									
Others: E. J. Pearce	Guest Worker	LPD, NIAID									
P. Brindley	Fogarty Fellow	LPD, NIAID									
COOPERATING UNITS (if any) George Washington University (S. L. James); Johns Hopkins University (M. Strand, J. Dalton); Cambridge University (A. E. Butterworth); Ffo Cruz/Belo Horizonte, Brazil (G. Gazzinelli).											
LAB/BRANCH Laboratory of Parasitic Diseases											
SECTION Immunology and Cell Biology Section											
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892											
TOTAL MAN-YEARS: 2.8	PROFESSIONAL: 2.0	OTHER: 0.8									
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews											
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The aim of this project is to study mechanisms of immunity and immune evasion in schistosomiasis with the ultimate goal of developing an experimental vaccine employing defined antigens.</p> <p>A. <u>Isolation of a protective vaccine antigen.</u> Monoclonal antibodies were raised against a 97 kD protein recognized monospecifically by mice vaccinated intradermally with schistosome extracts plus BCG. These monoclonals were used to purify the antigen which in preliminary experiments was shown to selectively elicit cell-mediated immunity (DTH, lymphokine production) in mice vaccinated with crude extracts. The anatomical localization and epitopic structure of the molecule were characterized and cellular and humoral responses to the antigen studied in infected or vaccinated mice as well as infected Kenyan and Brazilian patients.</p> <p>B. <u>Studies on the immune dependence of praziquantel therapy.</u> Through the use of μ-suppressed mice, the major anti-schistosomal drug praziquantel was shown to be dependent on the host humoral response in its <u>in vivo</u> effect on adult worms.</p>											

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00253-05 LPD	
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Studies of the Immunologic Responses to Filarial Infections		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: E. A. Ottesen Senior Investigator (Sen. I) LPD, NIAID Others: R. Hussain Senior Staff Fellow (Sen. SF)/Visiting Fellow (VF) LPD, NIAID T. B. Nutman Med. SF LPD, NIAID H. Francis Expert LIR, NIAID D. Ward Med. SF LPD, NIAID M. M. Frank Chief LCI, NIAID R. Davey Med. SF LCI, NIAID D. Freedman Med. SF LPD, NIAID R. Lal VF LPD, NIAID T. M. Chused Sen. I LI, NIAID		
COOPERATING UNITS (if any) Centre, Madras, India (S.P. Tripathy, P.R. Narayanan, V. Kumaraswami, R. Paranjape, K. Vijayasekaran, V. Vijayan); Onchocerciasis Chemotherapeut. Res. Centre, Tamale, Ghana (K. Awadzi, D. Badu); Special Prog. for Trop. Dis. Res., WHO, Geneva; CDC (C. Reimer); Dept. Health, Guatemala (G. Zea-Flores); Harvard Med. Sch. (S. Ackerman).		
LAB/BRANCH Laboratory of Parasitic Diseases		
SECTION Host Parasite Relations		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 5.1	PROFESSIONAL: 4.1	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The purpose of this project is to define the humoral and cellular immune responses that relate to immunopathology, protective immunity and immunodiagnosis of patients with lymphatic filariasis and onchocerciasis.</p> <p>Considerable effort has been directed towards developing monoclonal antibody reagents (Mcabs) for improving the sensitivity/specificity of several of the laboratory's important assays. Thus, antibodies of the IgG subclasses (which appear differentially important in blocking immediate hypersensitivity reactions [IgG₄] and in the pathogenesis of lymphatic lesions [IgG₃]) can be measured by well characterized and highly specific Mcabs; eosinophils can be identified by Mcabs directed against a unique surface antigen; their granule contents (MBP, EOP, EDN and ECP) are now assessable with affinity purified polyclonal and monoclonal antibodies; and the 200 kD circulating filarial antigen seen in infected patients can be measured much more sensitively with Mcabs directed against it or its immunodominant phosphorylcholine determinant.</p> <p>Studies of eye tissue and fluid from patients with ocular onchocerciasis show active local IgG and IgE antibody production as well as endothelial cell activation (Ia+) in the presence of microfilariae in the eye. T-cell infiltration of the iris and conjunctiva, however, is of the suppressor/cytotoxic phenotype (Leu 2a), opposite to the findings in most uveitis syndromes but consistent with the general parasite-specific, primary T-cell immunosuppression repeatedly demonstrated in both onchocerciasis and lymphatic filariasis.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00256-05 LPD
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Differentiation of <u>Leishmania</u> Promastigotes		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: D. Sacks		
Others: Roseangela da Silva Staff Fellow LPD, NIAID Steven Puentes Staff FELLOW LCI, NIAID		
COOPERATING UNITS (if any) Rajendra Memorial Institute, Patna, India (L. Prasad and staff)		
LAB/BRANCH		
SECTION		
INSTITUTE AND LOCATION		
TOTAL MAN-YEARS: 2.5	PROFESSIONAL: 2.5	OTHER:
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Metacyclic promastigotes are highly virulent to a susceptible vertebrate host. This virulence appears to be related to the ability of metacyclic forms to resist killing by the alternative complement pathway, and to their ability to resist the microbicidal activities of normal resident macrophages. A monoclonal antibody has been raised which recognizes a cell surface and secreted molecule which is unique to metacyclic promastigotes. It appears to be the exclusive molecule actively secreted by infective stage organisms. It is, therefore, likely to play a biologically critical role in promoting successful parasitism. Its chemistry and mode of action are being pursued.</p> <p>Approximately 2 months of each year are spent studying the immunology of visceral leishmaniasis in north India. Patients with active disease are specifically unresponsive at the T cell level to leishmanial antigens. This unresponsiveness cannot be reversed by depletion of T8 "suppressor" cells or by reconstitution with IL-2. We have recently detected a potentially large population of exposed, asymptomatic individuals from endemic areas based on their reactivity to parasite antigens <u>in vivo</u> and <u>in vitro</u>. The nature of the antigens recognized by the "immune" T cells is under study.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00257-05 LPD																																
PERIOD COVERED October 1, 1985 to September 30, 1986																																		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Immunology of Strongyloidiasis																																		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 45%;">F. A. Neva</td> <td style="width: 40%;">Chief</td> <td style="width: 20%;">LPD, NIAID</td> </tr> <tr> <td>Others:</td> <td>P. J. Brindley</td> <td>Visiting Fellow</td> <td>LPD, NIAID</td> </tr> <tr> <td></td> <td>W. T. London</td> <td>Section Chief, Experi. Pathology</td> <td>IRP, NINCOS</td> </tr> <tr> <td></td> <td>E. A. Ottesen</td> <td>Head, Clin. Parasitol.</td> <td>LPD, NIAID</td> </tr> <tr> <td></td> <td>T. B. Nutman</td> <td>Medical Staff Fellow</td> <td>LPD, NIAID</td> </tr> <tr> <td></td> <td>K. Barrett</td> <td>Visiting Fellow</td> <td>LCI, NIAID</td> </tr> <tr> <td></td> <td>D. Alling</td> <td>Spec. Asst./Biometry</td> <td>LCI, NIAID</td> </tr> <tr> <td></td> <td>T. Nash</td> <td>Senior Investigator</td> <td>LPD, NIAID</td> </tr> </table>			PI:	F. A. Neva	Chief	LPD, NIAID	Others:	P. J. Brindley	Visiting Fellow	LPD, NIAID		W. T. London	Section Chief, Experi. Pathology	IRP, NINCOS		E. A. Ottesen	Head, Clin. Parasitol.	LPD, NIAID		T. B. Nutman	Medical Staff Fellow	LPD, NIAID		K. Barrett	Visiting Fellow	LCI, NIAID		D. Alling	Spec. Asst./Biometry	LCI, NIAID		T. Nash	Senior Investigator	LPD, NIAID
PI:	F. A. Neva	Chief	LPD, NIAID																															
Others:	P. J. Brindley	Visiting Fellow	LPD, NIAID																															
	W. T. London	Section Chief, Experi. Pathology	IRP, NINCOS																															
	E. A. Ottesen	Head, Clin. Parasitol.	LPD, NIAID																															
	T. B. Nutman	Medical Staff Fellow	LPD, NIAID																															
	K. Barrett	Visiting Fellow	LCI, NIAID																															
	D. Alling	Spec. Asst./Biometry	LCI, NIAID																															
	T. Nash	Senior Investigator	LPD, NIAID																															
COOPERATING UNITS (if any) SEMA, Inc., Rockville, MD (G. Phillips); Merck, Sharpe, Dohme; Rahway, N.J. (Kenneth Brown, M.D.).																																		
LAB/BRANCH Laboratory of Parasitic Diseases																																		
SECTION Cell Biology and Immunology Section																																		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205																																		
TOTAL MAN-YEARS: 1.1	PROFESSIONAL: 0.7	OTHER: 0.4																																
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews																																		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> This project involves clinical and immunologic studies of humans infected with the intestinal nematode <u>Strongyloides stercoralis</u>, and similar studies in an experimental host, the <u>Patas</u> monkey. Parasite antigens are prepared from larvae derived from chronic infections in the <u>Patas</u> monkey. During the past year most of the effort has been on studies of third stage larval antigens by iodination of surface proteins and metabolic labeling of both somatic and metabolic antigens with ³⁵S-methionine. At least 16 surface proteins, ranging from MW of 200 to < 6 K in size, could be identified by SDS polyacrylamide gel electrophoresis after iodination. A loss of one surface protein and the appearance of another was demonstrated in larvae after penetration of rat skin. About 30 components labeled with ³⁵S-methionine were found in larval somatic antigens, but only about ten proteins, mainly 50 to 70 K in MW size, were found in the metabolic products of larvae by the same method. Many of these antigens could be immunoprecipitated with a pool of human serum from infected patients. Observations continue to be made on immediate skin test reactions to somatic and metabolic larval antigens in patients with strongyloidiasis, as well as patients with other nematode infections. Examples of the greater specificity of the metabolic skin test antigen were seen in patients with filarial infections. Several patients with strongyloides infection were treated with a new drug, Ivermectin. </p>																																		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00258-04 LPD												
PERIOD COVERED October 1, 1986 to September 30, 1986														
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Development of Models for Chagas' Disease Using T. cruzi Clones and Inbred Mice														
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: M. Postan</td> <td style="width: 33%;">WHO/Visiting Associate</td> <td style="width: 33%;">LPD, NIAID</td> </tr> <tr> <td>Others: J.A. Dvorak</td> <td>Res. Microbiologist</td> <td>LPD, NIAID</td> </tr> <tr> <td>J. Bailey</td> <td>Chief, Med. Application Sect.</td> <td>IAS, DCRT</td> </tr> <tr> <td>E. Pottala</td> <td>Senior Engineer</td> <td>IAS, DCRT</td> </tr> </table>			PI: M. Postan	WHO/Visiting Associate	LPD, NIAID	Others: J.A. Dvorak	Res. Microbiologist	LPD, NIAID	J. Bailey	Chief, Med. Application Sect.	IAS, DCRT	E. Pottala	Senior Engineer	IAS, DCRT
PI: M. Postan	WHO/Visiting Associate	LPD, NIAID												
Others: J.A. Dvorak	Res. Microbiologist	LPD, NIAID												
J. Bailey	Chief, Med. Application Sect.	IAS, DCRT												
E. Pottala	Senior Engineer	IAS, DCRT												
COOPERATING UNITS (if any) None														
LAB/BRANCH Laboratory of Parasitic Diseases														
SECTION Physiology and Biochemistry														
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892														
TOTAL MAN-YEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0												
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews														
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) Project terminated.														

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00347-04 LPD
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Schistosomal Hepatic Fibrosis		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: A. W. Cheever Assistant Chief LPD, NIAID		
Others: R. H. Duvall Bio. Lab. Tech. (Micro.) LPD, NIAID J. Malley Mathematical Statistician LSM, DCRT A. Sher Head, Immunology Section LDP, NIAID R. Minker LSM, DCRT		
COOPERATING UNITS (if any) Brigham & Women's Hospital, Boston, Mass., Dept. of Pathology (F. V. Lichtenberg, J. Bryam)		
LAB/BRANCH Laboratory of Parasitic Diseases		
SECTION Host-Parasite Relations Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205		
TOTAL MAN-YEARS: 1.5	PROFESSIONAL: 0.75	OTHER: 0.75
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> Hepatic fibrosis is examined in mice infected with schistosome species pathogenic for man. Mouse strains developed markedly different degrees of hepatic fibrosis following infection with <u>S. mansoni</u>. T cells are important for the formation of granulomas in both <u>S. mansoni</u> and <u>S. japonicum</u> infected mice, as determined from examination of <u>athymic</u> and <u>B cell depleted</u> mice. Current studies are focused on determining which subsets of T cells are involved in regulation of <u>S. japonicum</u> egg granulomas. BALB/c mice are not much more responsive to <u>S. japonicum</u> eggs than are BALB/c nu/nu mice, precluding studies of cell transfer in this strain. BALB/c mice do have more fibrosis than do their nude counterparts, emphasizing the role of T cells in hepatic fibrosis in this system and providing one more example of the dissociation of granuloma size and fibrosis. T cells are required for the formation of normal granulomas, as indicated by marked differences between NCR outbred nu/nu versus nu/+ and between C57BL/6 nu/nu versus C57BL/6. Studies of cell transfer from normal into nude mice have begun with this last combination. The intensity of murine <u>S. mansoni</u> infection was found to modulate hepatic pathology. With increasing intensity of infection a smaller proportion of eggs was found in the liver and less fibrosis per egg was noted. The volume of hepatic granulomas, in contrast to <u>S. japonicum</u> infected mice, did not vary with infection intensity. The dissociation of the effects of infection intensity on fibrosis and granuloma volume indicates that they are under different regulatory influences. The fecundity of <u>S. mansoni</u> worm pairs decreased with increasing intensity of infection, again in contrast to <u>S. japonicum</u> infected mice. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00348-04 LPD																					
PERIOD COVERED October 1, 1985 to September 30, 1986																							
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Immunity in Murine Schistosomiasis																							
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: A. W. Cheever</td> <td style="width: 33%;">Assistant Chief</td> <td style="width: 33%;">LPD, NIAID</td> </tr> <tr> <td colspan="3">Others:</td> </tr> <tr> <td>R. H. Duvall</td> <td>Bio. Lab. Tech. (Micro)</td> <td>LPD, NIAID</td> </tr> <tr> <td>A. Sher</td> <td>Section Head</td> <td>LPD, NIAID</td> </tr> <tr> <td>J. Malley</td> <td>Mathematical Statistician</td> <td>LSM, DCRT</td> </tr> <tr> <td>K. Malley</td> <td>Computer Assistant Analyst</td> <td>LSM, DCRT</td> </tr> <tr> <td>P. Shade</td> <td>Biol Lab. Tech.</td> <td>LPD, NIAID</td> </tr> </table>			PI: A. W. Cheever	Assistant Chief	LPD, NIAID	Others:			R. H. Duvall	Bio. Lab. Tech. (Micro)	LPD, NIAID	A. Sher	Section Head	LPD, NIAID	J. Malley	Mathematical Statistician	LSM, DCRT	K. Malley	Computer Assistant Analyst	LSM, DCRT	P. Shade	Biol Lab. Tech.	LPD, NIAID
PI: A. W. Cheever	Assistant Chief	LPD, NIAID																					
Others:																							
R. H. Duvall	Bio. Lab. Tech. (Micro)	LPD, NIAID																					
A. Sher	Section Head	LPD, NIAID																					
J. Malley	Mathematical Statistician	LSM, DCRT																					
K. Malley	Computer Assistant Analyst	LSM, DCRT																					
P. Shade	Biol Lab. Tech.	LPD, NIAID																					
COOPERATING UNITS (if any) Biomedical Research Institute, Rockville, MD (F. Lewis and C. Richards) George Washington University (S. James)																							
LAB/BRANCH Laboratory of Parasitic Diseases																							
SECTION Host-Parasite Relations Section																							
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205																							
TOTAL MAN-YEARS: 1.25	PROFESSIONAL: 0.25	OTHER: 1.0																					
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td><input type="checkbox"/> (a) Human subjects</td> <td><input type="checkbox"/> (b) Human tissues</td> <td><input checked="" type="checkbox"/> (c) Neither</td> </tr> <tr> <td><input type="checkbox"/> (a1) Minors</td> <td></td> <td></td> </tr> <tr> <td><input type="checkbox"/> (a2) Interviews</td> <td></td> <td></td> </tr> </table>			<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither	<input type="checkbox"/> (a1) Minors			<input type="checkbox"/> (a2) Interviews														
<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither																					
<input type="checkbox"/> (a1) Minors																							
<input type="checkbox"/> (a2) Interviews																							
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Substrains of <u>S. mansoni</u> selected for varying infectivity to vector snails have been tested for their ability to induce <u>immunity in mice</u>. Two strains derived from the same patients and designated PRT-3 and PRC-3 induced markedly different degrees of <u>resistance to infection</u> after a bisexual first injection. The F-1 <u>cross</u> between these strains also produced high resistance, comparable to that induced by the "immunogenic" PRT-3 strain. C57BL/KsJ mice with <u>unisexual S. mansoni</u> infections generally were about 40% resistant to challenge infections; however, the PCR-3 strain induced no resistance after unisexual infection.</p> <p>Although these results were repeated several times over a 3 year period, we have not been able to consistently repeat them in the past year. This nullifies any attempt to analyse the mechanisms which may have been involved and the project is being discontinued. There is some indication that the water injected with the schistosome cercariae may itself have some effect on the resistance of mice and we will reexamine this point before discontinuing the project.</p>																							

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00350-04 LPD
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) DNA Analysis of Parasites		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: T. E. Nash Medical Officer LPD, NIAID Others: A. Aggarwal Visiting Fellow Rod Adam Medical Staff Fellow A. Lal Guest Researcher T. McCutchen Senior Scientist P. Romans Staff Fellow		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Parasitic Diseases		
SECTION Host-Parasite Relations Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205		
TOTAL MAN-YEARS 2.2	PROFESSIONAL: 1.7	OTHER: 0.5
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Nine new isolates of <u>Giardia</u> have been axenized and are in the process of being studied. The DNA of other isolates were analyzed by endonuclease restriction analysis and found to be different from most of the previously defined <u>Giardia</u> isolates. In order to study the structure and gene organization of the major 170kD antigen of isolate WB, the gene was cloned into an expression vector and a number of clones isolated. Sequencing of this gene is in progress. The insert from this vector was cloned into M13 and used as a probe in restriction endonuclease studies of various isolates. The gene is present in isolates that do not express the 170kD antigen.</p> <p>Mutants of <u>Giardia</u> could not be produced by commonly used procedures. Transfection of <u>Giardia</u> was attempted with Icarus hs neo gene which confers resistance to G418. Although RNA transcripts were found, no detectable translated products were found. The heat shock gene (hs-p) of <u>Giardia</u> was isolated and cloned into Puc18 and the promotor for the gene will be used with the neo gene.</p> <p>Translation products of RNAs from different isolates showed subtle but definite differences. A 43kD product was recognized by infected human and gerbil sera.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00351-04 LPD
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Parasite and Host Factors Controlling the Pathogenesis of Leishmaniasis		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: P. A. Scott Senior Staff Fellow LPD, NIAID		
Others: A. Sher Section Head LPD, NIAID F. A. Neva Chief LPD, NIAID P. Natovitz Biologist LPD, NIAID D. Dwyer Supvr. Microbiologist LPD, NIAID E. Pearce Guest Researcher LPD, NIAID		
COOPERATING UNITS (if any) Wellcome Research Laboratories, Experimental Biology Division, London, England (J. Howard); George Washington University, Washington, DC (S. James).		
LAB/BRANCH Laboratory of Parasitic Diseases		
SECTION Immunology and Cell Biology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205		
TOTAL MAN-YEARS: 2.5	PROFESSIONAL: 1.5	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Leishmaniasis is a chronic protozoal disease of man associated with both protective and immunopathologic immune responses. To define these responses, a vaccine against <u>Leishmania major</u> was developed in which protective immunity is induced in BALB/c mice by intraperitoneal immunization with a soluble fraction of promastigotes. To identify functional immunogens, the soluble antigen preparation was passed over an FPLC Mono Q column, and separated into 9 pools. We then assessed T cell responses, including lymphocyte transformation, IL-2 production, delayed hypersensitivity and macrophage activating factor (MAF) production, as well as antibody responses, to each pool. Although several pools were capable of stimulating T cells only one pool induced significant protection. The protective pool contains a group of highly negatively charged molecules, including both proteins and DNA. Enzymatic removal of protein destroyed the protective activity of the fraction, while digestion of DNA had no effect. Several proteins were unique to this pool, including a predominant 35Kd antigen. Monoclonal antibodies have been raised against these unique molecules and will be used to affinity purify potentially protective antigens. These results suggest that only a small subset of the T cells stimulated by leishmanial antigens induce protection. We are presently investigating the possibility that the non-protective T cells may induce immunopathologic responses. Finally, we have studied non-healing <u>L. major</u> infections in two other mouse models. The P/J mouse strain fails to heal following infection, and it has been suggested that the failure to heal is due to a qualitative defect in MAF production. We overcame this defect by vaccination, as well as in vivo removal of a subset of regulatory T cells, suggesting that the defect in MAF production is due to a regulatory T cell imbalance. We have also shown that B cell deficient C3H/HeN mice fail to heal normally, and found that this may be due to the lack of a T cell subset that requires B cells for its normal maturation.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00439-02 LPD
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Clinical and Therapeutic Studies in Human Filariasis		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: E. A. Ottesen Senior Investigator LPD, NIAID		
Others: T. B. Nutman Medical Staff Fellow LPD, NIAID D. Ward Medical Staff Fellow LPD, NIAID B. Curry Visiting Associate LPD, NIAID R. Davey Medical Staff Fellow LCI, NIAID		
COOPERATING UNITS (if any) PB, NHLBI, NIH (R. G. Crystal); Tuberculosis Research Centre, Madras, India; (R. Prabhakar and V. Kumaraswami); Madras Medical Mollege, Madras, India (K. Vijayasekaran); Peace Corps Medical Office (K. D. Miller and M. Mulligan).		
LAB/BRANCH Laboratory of Parasitic Diseases		
SECTION Host Parasite Relations		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> Trials of two new drugs for bancroftian filariasis have been initiated with collaborators in Madras, India. Ivermectin (Phase IIa) at doses between 25-200 mcg/kg once orally has been found to be a rapid, extremely effective microfilaricide, but by three months the microfilarial load has returned to ~ 10% of pre-treatment levels. Therefore its adult-filaricidal potential requires additional evaluation as does the importance of side reactions associated with the rapid death (first 12-24 hours) of the microfilariae. CGP 20376 (Phase I) has been successfully given to normals in doses from 0.01-1.0 mg/kg once orally without any significant side effects. Its filaricidal activity in man must now be evaluated. </p> <p> Because long-term follow-up studies employing bronchoalveolar lavage to evaluate patients treated conventionally with diethylcarbamazine (DEC) have shown that < 1/3 relapse or have persistent low-grade alveolitis leading to pulmonary fibrosis, a therapeutic trial of three regimens (conventional DEC, long-term DEC, or DEC + steroids) has been initiated in Madras. The study will require about three years to enroll the anticipated 75 study patients. </p> <p> Observations on 200 Peace Corps volunteers going to <u>Loa loa</u> endemic areas of Africa are concluding after two years of a placebo-controlled chemoprophylaxis trial using weekly doses of DEC. Seroconversion is at ~ 15%, but the placebo <u>vs.</u> drug code will not be broken until October, 1986, so that the success of the prophylactic regimen cannot yet be determined. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 AI 00483-01 LPD
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Plasmodium Chromosome Organization & Genomic Restructuring in Response to Stress		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Thomas E. Wellem's Others: Louis H. Miller Thomas F. McCutchan Russell J. Howard Lindsey J. Panton Richard Carter		
COOPERATING UNITS (if any) Biomedical Research Institute, Rockville, MD (Virgilio Rosario); WRAIR, Washington, DC (Ayo Odoula); Columbia University, New York (Cassandra Smith, Charles Cantor); Rockefeller University, New York (David Irving, Joan Ellis, George A.M. Cross); David Walliker, Edinburgh, Scotland.		
LAB/BRANCH Laboratory of Parasitic Diseases		
SECTION Malaria Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard un-reduced type. Do not exceed the space provided.) New and irreversible modifications of genomic structure are induced in <u>Plasmodium</u> when the malaria parasite is subjected to stress. These modifications give rise to altered patterns of gene expression which modulate the parasite phenotype. Conditions known to induce genomic changes include drug pressure, growth environments imposed by <u>in vitro</u> culture, cross-fertilization between different parasite genomes, and antibody pressure. We now have the capability to enumerate, size and map chromosomes of <u>Plasmodium</u> . We are working to characterize the details of chromosome organization and genomic alterations induced by stress.		

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
66
67
68
69
70
71
72
73
74
75
76
77
78
79
80
81
82
83
84
85
86
87
88
89
90
91
92
93
94
95
96
97
98
99
100



10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
66
67
68
69
70
71
72
73
74
75
76
77
78
79
80
81
82
83
84
85
86
87
88
89
90
91
92
93
94
95
96
97
98
99
100

LABORATORY OF VIRAL DISEASES
1986 ANNUAL REPORT
Table of Contents

SUMMARY	12-1
Project Number	
Z01 AI 00020-11 LVD	Studies on the Treatment of Disease with the Interferon System - Levy.....12-7
Z01 AI 00123-20 LVD	Structure and Replication of Poxvirus DNA - Merchlinsky.....12-8
Z0I AI 00126-13 LVD	Functional Analysis of Poxvirus Genome - DeFilippes.....12-9
Z01 AI 00298-05 LVD	Development of Vaccinia Virus as an Expression Vector - Moss12-10
Z01 AI 00306-05 LVD	Pathogenesis of Orthopoxvirus Infection - Buller..12-11
Z01 AI 00307-05 LVD	Regulation of Vaccinia Virus Gene Expression - Moss.....12-12
Z01 AI 00391-03 LVD	Expression of Genes of Respiratory Viruses by Vaccinia Virus Recombinants - Elango.....12-13
Z01 AI 00392-03 LVD	Expression of Hepatitis B Virus Genes by Vaccinia Virus Recombinants - Brechling.....12-14
Z01 AI 00393-03 LVD	Expression of Malaria Genes by Vaccinia Virus Recombinants - Moss.....12-15
Z01 AI 00416-03 LVD	Recombinant Vaccines Against Retroviruses Associated with Leukemia and AIDS - Chakrabarti...12-16
Z0I AI 00443-02 LVD	Vaccinia Virus Growth Factor - Buller.....12-17
Z01 AI 00444-02 LVD	Regulation of Expression of Herpes Simplex Virus Genes - Weir.....12-18
Z01 AI 00445-02 LVD	Mechanisms of Viral DNA Replication - Challberg...12-19

11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
66
67
68
69
70
71
72
73
74
75
76
77
78
79
80
81
82
83
84
85
86
87
88
89
90
91
92
93
94
95
96
97
98
99
100

101
102
103
104
105
106
107
108
109
110
111
112
113
114
115
116
117
118
119
120
121
122
123
124
125
126
127
128
129
130
131
132
133
134
135
136
137
138
139
140
141
142
143
144
145
146
147
148
149
150
151
152
153
154
155
156
157
158
159
160
161
162
163
164
165
166
167
168
169
170
171
172
173
174
175
176
177
178
179
180
181
182
183
184
185
186
187
188
189
190
191
192
193
194
195
196
197
198
199
200

PHS-NIH
SUMMARY REPORT

ANNUAL REPORT OF THE LABORATORY OF VIRAL DISEASES, NIAID
October 1, 1985 to September 30, 1986

Dr. Bernard Moss
Chief, Laboratory of Viral Diseases

The Laboratory of Viral Diseases carries out a program of fundamental investigations on the molecular biology of viruses, the interactions of viruses with host cells, the pathogenesis of viral diseases, and host defense mechanisms. Current topics of research include: regulation of gene expression, mechanisms of DNA replication, virus growth factors, development of recombinant expression vectors, factors determining virus virulence, host resistance genes, targets of cell-mediated immunity, and genetic engineering of live recombinant vaccines.

Highlights of this year's research efforts are summarized below.

REGULATION OF VACCINIA VIRUS GENE EXPRESSION

Vaccinia virus has a genome of 185,000 bp that encodes approximately 200 polypeptides, including a multisubunit RNA polymerase, capping and methylating enzymes, and poly(A) polymerase. These genes are expressed in a coordinated fashion so that some polypeptides are made before and others after DNA replication. In order to learn more about regulation of late gene expression, an 8,600 base pair region of the vaccinia genome was sequenced. Seven complete open-reading-frames were found. Six transcripts that initiated within this region were detected and of these, four were synthesized only at late times. In each case, the transcriptional and translational start sites mapped within a few nucleotides of each other and the sequence TAAATG occurred at the start of the open-reading-frame. The extremely short leader and the absence of A or G in the -3 position, relative to the first nucleotide of the initiation codon, distinguishes the majority of vaccinia virus late genes from eukaryotic and vaccinia virus early genes (Rosel, Weir, and Moss).

The putative promoter region of one late gene was analyzed by linking DNA segments to the coding region of the prokaryotic enzyme chloramphenicol acetyltransferase and recombining this construct into the vaccinia virus genome. By repeating this procedure with smaller and smaller putative promoter segments and assaying for enzyme activity, the location of the regulatory signal was determined. Mutants that retained only 18 base pairs of DNA before and ten base pairs after the RNA start site still had a functional, although weakened, promoter. Further 5' to 3' deletions, however, resulted in loss of activity (Weir and Moss).

An in vitro transcription system that accurately initiates and terminates transcription of early vaccinia virus genes has been prepared from purified infectious vaccinia virus particles. Using this system, the signal for termination has been localized about 50 base pairs upstream of the actual site of termination (Yuen and Moss).

An RNA polymerase complex that carries out faithful transcription of early vaccinia virus genes was isolated by glycerol gradient sedimentation. Two other enzymes, a DNA-dependent ATPase and a capping/methylating enzyme, were physically associated with the transcription complex. Further evidence that the DNA-dependent ATPase is required was obtained through the use of nucleotide analogs (Broyles and Moss).

REGULATION OF HERPESVIRUS LATE GENE EXPRESSION

Herpes simplex virus 1 (HSV-1) is a large double-stranded DNA virus whose gene products are sequentially regulated in a cascade fashion. Immediate early genes are transcribed in the absence of de novo protein synthesis soon after viral infection. A second class of genes are expressed before viral DNA synthesis, but required previous synthesis of immediate early genes. The expression of late genes is dependent upon or greatly enhanced by DNA replication. The expression of glycoprotein C, a late gene, was studied by ligating the putative promoter region of this gene to the coding sequence for bacterial β -galactosidase and recombining the construct into the HSV-1 genome. By making deletions in the putative gC promoter, the regulatory region was shown to lie within 104 bp upstream of the RNA start site (Weir and Narayanan).

STRUCTURE AND REPLICATION OF POXVIRUS DNA

Poxviruses provide a unique experimental system for studying DNA replication. The ends of the linear double-stranded DNA genome consist of hair-pin structures that may resemble telomeres of eukaryotic chromosomes. Enzymes and other proteins needed for DNA synthesis are encoded within the viral genome and replication occurs in the cytoplasmic compartment of infected cells. During the previous year, the DNA polymerase gene was sequenced. Drug resistant DNA polymerase mutants are now being analyzed in order to locate the active sites of the enzyme.

Marker transfer experiments indicated that a mutation conferring resistance to phosphonoacetate was located in a 2,000 base pair segment of the DNA polymerase gene. Nucleotide sequencing revealed only two nucleotide substitutions. In order to determine whether one or both of these changes are required for drug resistance, oligonucleotide mutagenesis was performed. Marker transfer experiments demonstrated that only one of these changes, producing a predicted glycine to aspartic acid change at amino acid 347, was necessary. Since phosphonoacetate is an analog of pyrophosphate, this mutation may signify a part of the catalytic site of DNA polymerase (Earl and Moss).

Another mutation, conferring resistance to the drug aphidicolin, also was mapped within the DNA polymerase gene. The mutated site was located within a 124 base pair DNA fragment by marker transfer and a single nucleotide substitution predicting a leucine to methionine change was found. Interestingly, the aphidicolin resistant site is 892 bases from the site of phosphonoacetate resistance (DeFilippes).

Evidence has been obtained that the hairpin telomeres of vaccinia virus genomic DNA can arise by resolution of the junction region of concatemers. This was shown by the conversion, in vaccinia virus infected

cells, of circular plasmids containing concatemer junctions to linear minichromosomes with hairpin ends. A DNA segment required for this resolution reaction was identified by deletion mutagenesis (Merchinsky and Moss).

VIRAL GENES REQUIRED FOR HERPES SIMPLEX DNA REPLICATION

The mechanism of herpes simplex virus (HSV) DNA replication is not well understood. Previous genetic methods were used to show that three genes -- DNA polymerase, a single-stranded DNA binding protein, and ribonucleotide reductase -- are involved in DNA synthesis. During the past year, a transfection assay was developed that has allowed the localization of eight viral genes that are required for replication of plasmids containing HSV origins of replication. Thus, at least five additional, previously unidentified replication genes have been identified (Wu and Challberg).

VACCINIA VIRUS GROWTH FACTOR

Vaccinia virus has recently been shown to encode a protein (VGF) that is related to epidermal and transforming growth factors. This viral protein is secreted from infected cells, binds to epidermal growth factor receptors, and stimulates cell growth. To determine the biological role of VGF, vaccinia virus mutants were constructed with deletions corresponding to the active site of the gene. The mutant virus grows normally in tissue culture cells but fails to induce a cell proliferative response in some. Similarly, the mutant virus produced pocks on the chorioallantoic membrane of embryonated eggs but induced a much lower cell proliferative response and a lower yield of virus. It seems likely, then, that the role of VGF is to induce rapidly-dividing cells that are susceptible to virus infections (Buller and Moss).

PATHOGENESIS OF ORTHOPOXVIRUS INFECTIONS

The pathogenesis of orthopoxvirus infection is determined by both virus and host factors. Using ectromelia virus and two separate experimental approaches, it was shown that recovery from a primary infection did not require the synthesis of anti-ectromelia virus neutralizing antibody. These experiments also showed that a normal anti-ectromelia CTL response could be generated in the absence of L3T4⁺ ("helper" T-cells). This CTL maturation pathway may be of general importance in resistance to a variety of agents that replicate in cells and express antigens at the cell surface. Genetic analysis of crosses between susceptible (A/J) and resistant (C57BL/6J) mice indicated that at least two non-H2 genes were important in the recovery of mousepox. For vaccinia virus, which is very closely related to ectromelia, the vaccinia virus growth factor (VGF) gene was shown to be important for virulence. In BALB/cByJ mice, the LD₅₀ of VGF mutant virus was 100-fold higher than that of parental virus (Buller).

STUDIES WITH POLY ICLC, AN IMMUNE MODULATOR

Further studies have reinforced the conclusion that the optimally effective concentration of poly ICLC may be considerably lower than that used in previous experimental animal and clinical trials. For example, in monkeys, only low doses of poly ICLC produce an elevation in lymphocytes

and NK cells. Analysis of data from three clinical studies indicates that poly ICLC causes a small but regular increase in T helper to T suppressor ratios in addition to enhancing macrophage activity in most patients and NK activity in some. Other collaborative studies indicate that the drug may have prophylactic and therapeutic value for Rift Valley Fever (Levy).

DEVELOPMENT OF VACCINIA VIRUS AS AN EXPRESSION VECTOR

A new eukaryotic transient expression system based on recombinant vaccinia virus that synthesizes bacteriophage T7 RNA polymerase has been developed. This new system is about 500-fold more efficient than previous transient expression systems that rely on the enhancer and promoter elements of the long terminal repeat of Rous sarcoma virus or the early region of SV40. It is anticipated that this vector system will find wide application (Fuerst and Moss).

DETERMINATION OF THE TARGETS OF CYTOTOXIC T CELLS (CTL)

Recently, it has been shown that the nucleoprotein (NP) of influenza virus is a major target of mouse CTL. The importance of non-envelope proteins as CTL targets was extended this past year by using vaccinia virus vectors to demonstrate that influenza NP is also a target of human CTL. Furthermore, the nucleocapsid protein of vesicular stomatitis virus was shown to be a major CTL target (Moss).

PROTECTION OF EXPERIMENTAL ANIMALS AGAINST RABIESVIRUS

Several new recombinant vaccinia viruses that express the rabiesvirus glycoprotein were constructed. Cells infected with each recombinant virus made a protein that reacted with antibody to G, comigrated with authentic G and was transported to the plasma membrane. The highest amounts of G were made when the promoter, RNA start site, and translation initiation codon all were obtained from the gene for the vaccinia virus 11 kD structural protein. Upon vaccination of mice, each recombinant virus induced production of rabiesvirus neutralizing antibodies and protection against lethal intracerebral rabiesvirus challenge (Brechling and Moss).

PROTECTION OF EXPERIMENTAL ANIMALS AGAINST HERPES SIMPLEX VIRUS

In previous years we demonstrated that recombinant vaccinia viruses that express HSV-1gD can be used to protectively immunize mice against the development of lethal and latent HSV-1 infections. Further studies indicate that the protection is very durable, lasting for at least one year after inoculation. The recombinant vaccinia virus also was used to show that HSV-1gD is a target for human cytotoxic T cell clones (Moss).

PROTECTION OF EXPERIMENTAL ANIMALS AGAINST RESPIRATORY SYNCYTIAL VIRUS

Recombinant vaccinia viruses that express either the G or F proteins of respiratory syncytial virus (RSV) were constructed. In each case, the proteins appeared to be synthesized and processed normally. Cotton rats that were vaccinated with either recombinant virus made RSV neutralizing antibodies and were resistant to lower respiratory challenge with RSV (Elango and Moss).

CHARACTERIZATION AND IMMUNOGENICITY OF HEPATITIS B VIRUS SURFACE PROTEINS CONTAINING PRE-S PEPTIDES

Recent studies indicate that the pre-S region of the hepatitis B virus surface protein is highly immunogenic and may be important to include in vaccines. Two new recombinant vaccinia viruses that express the pre-S1, pre-S2 and S regions or only the pre-S2 and S region were constructed. Cells infected with the latter virus synthesized and secreted a novel middle S protein particle, whereas the large S protein synthesized by the former virus was not secreted. Nevertheless, rabbits inoculated with either recombinant virus produced antibodies to both pre-S and S epitopes (Cheng and Moss).

THE IMMUNOGENICITY OF A MALARIAL PROTEIN IS ENHANCED BY GENETIC ENGINEERING

A recombinant vaccinia virus that expressed a secreted plasmodial antigen with repeated epitopes induced a very low antibody response in rabbits and mice. By genetic engineering, the transmembrane domains of an IgG1 was attached to the carboxyl end of the malaria protein. Now the malaria protein was anchored in the membrane of cells infected with the recombinant virus, and inoculated animals produced an enhanced antibody response. Other studies indicated that vaccinia virus recombinants that express the plasmodial circumsporozoite antigen, which also has repeated epitopes, induce antibodies in experimental animals. This malaria protein is neither secreted nor expressed on the surface of vaccinia infected cells (Cheng and Moss.)

T-LYMPHOCYTE PRIMING AND PROTECTION AGAINST FRIEND MURINE LEUKEMIA BY A RECOMBINANT VACCINIA VIRUS THAT EXPRESSES THE RETROVIRUS ENVELOPE GENE

The envelope (env) gene from Friend murine leukemia virus (F-MuLV) was inserted into the genome of a vaccinia virus expression vector. Infected cells synthesized gp85, the glycosylated primary product of the env gene. Processing to gp70 and p15E, and cell surface localization, were similar to that occurring in F-MuLV infected cells. Mice that were inoculated with the live recombinant vaccinia virus had an envelope-specific T cell proliferative response and following challenge with FV complex developed neutralizing antibody and cytotoxic T cells (CTL) and were protected against leukemia (Earl and Moss).

SURFACE EXPRESSION OF RETROVIRAL GLYCOPROTEINS IS POLARIZED IN EPITHELIAL CELLS INFECTED WITH RECOMBINANT VACCINIA VIRUS

Murine leukemia virus buds from the basolateral surface of polarized epithelial cells. The Friend virus envelope protein, made by a recombinant vaccinia virus in MDCK cells, was found only on the basolateral surface, indicating that no other retrovirus proteins are required for polarized transport (Earl and Moss).

EXPRESSION OF AIDS VIRUS ENVELOPE PROTEIN BY A RECOMBINANT VACCINIA VIRUS

In tissue culture cells infected with a recombinant vaccinia virus, the glycosylated gp160 envelope precursor was synthesized and processed to form the gp120 and gp41 mature subunits. Mice vaccinated with the recombinant virus made antibodies to the AIDS virus envelope protein (Chakrabarti and Moss).

ADMINISTRATIVE CHANGES

Several members of the laboratory completed their postdoctoral training and accepted positions at other institutions. Dr. Jerry Weir will be moving to the University of Tennessee as an Assistant Professor; Dr. K.-C. Cheng will leave for Cornell University Medical School as Assistant Professor; Dr. S. Elango left for the National Institute of Virology in Poona India, as Deputy Director; Dr. S. Chakrabarti moved to the Immunology Institute in Delhi, India, as a Research Scientist; and Dr. P. Narayanan returned to the Tuberculosis Research Centre in Madras, India, as Assistant Director of the Department of Immunology.

New members of LVD include Dr. Carol Wu, Staff Fellow; Dr. Stewart Shuman, Medical Staff Fellow; Dr. Paul Olivo, Medical Staff Fellow; Dr. Falko Falkner, Guest Researcher; and Dr. Ambrose Hugins, Guest Researcher. Dr. Patricia Earl was promoted to a permanent position as Supervisory Microbiologist within LVD.

HONORS AND AWARDS

Dr. Moss continues to serve on the editorial boards of the Journal of Virology, Virology, and Journal of Biological Chemistry and on the advisory board of Advances in Virus Research. He completed a four year term on the Advisory Committee on Nucleic Acid and Protein Synthesis of the American Cancer Society, and served as an advisor to the World Health Organization and to other U.S. government agencies, and is on the Board of Trustees of FAES. Dr. Moss was selected as one of the 100 most innovative scientists of 1985 by Science Digest, received an Inventors Award from the U.S. Department of Commerce, received the U.S.P.H.S. Distinguished Service Medal and delivered the Stanhope Bayne-Jones lecture at Johns Hopkins University School of Medicine.

Dr. Levy is an editor of the Journal of Bioactive Polymers and the Journal of Biological Regulators and Homeostatic Agents.

Members of LVD presented numerous invited lectures in this country and abroad.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00020-11 LVD
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Studies on the Treatment of Disease with the Interferon System		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: H.B. Levy Section Head LVD, NIAID		
COOPERATING UNITS (if any) NCI, (A. Maluish); PRI (contractor to NCI, J. Talmadge); Genessee Hospital, Rochester, NY, (E. Lvovsky); USAMRIID, (M. Kende and P. Canonico); Walter Reed, (A. Salazar); NINCDS, (D. McFarlin); U. Texas Med. Sch., (R. Tyndall); CDC, (G. Bagar).		
LABORATORY Laboratory of Viral Diseases		
SECTION Molecular Virology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 1	PROFESSIONAL: 1	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> Toward the end of the period covered in last year's annual report a conclusion of a meeting on poly ICLC held by the NCI was that previous clinical studies have used much too high a level of drug. During the past year we have begun investigating the effects of low doses of poly ICLC on several immune parameters. The details of the type of immune modulation seen appear to be quite dependent on dose level. For example, at very low levels in monkeys, there is seen an increase in % lymphocytes with no change in total WBC, at levels 10X as high (but still lower than we had been using clinically), there is a decrease in % lymphocytes and in total WBC. At the low levels there is activation of both NK cells and macrophage activity, but at the somewhat higher levels, NK activity is decreased, while macrophage activity is still enhanced. The lower level (0.2 mg/m²) is too low to elude detectable serum interferon; the higher one induces 50-100 µAml of serum. </p> <p> Analysis of data from 3 clinical studies indicates that there is a small, but regular increase in T helper to T suppressor ratio, in addition to enhancing macrophage activity in most patients, and NK activity in a smaller fraction. More detailed studies of these immune alterations in cancer patients, as a function of dose, route and schedule are planned. </p> <p> Studies with USAMRIID, using a virus of military importance Rift Valley Fever Virus, have shown that the drug is effective in protecting against infection both prophylactically and therapeutically, and as an adjuvant with RVFV vaccine. Clinical studies with USAMRIID are being discussed, using poly ICLC as an adjuvant with a vaccine, and as a therapeutic agent in volunteers with a self limiting virus. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00123-20 LVD
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Structure and Replication of Poxvirus DNA		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: M. Merchlinsky Staff Fellow LVD, NIAID		
Others: B. Moss Laboratory Chief LVD, NIAID		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Viral Diseases		
SECTION Macromolecular Biology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205		
TOTAL MAN-YEARS: 1.45	PROFESSIONAL: 1.1	OTHER: 0.35
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided) <p>Poxviruses provide a unique experimental system for studying DNA replication. The ends of the linear double-stranded DNA genome consist of hair-pin structures that may resemble telomeres of eukaryotic chromosomes. Enzymes and other proteins needed for DNA synthesis are encoded within the viral genome and replication occurs in the cytoplasmic compartment of infected cells. During the previous year the DNA polymerase gene of vaccinia virus was completely sequenced and the primary structure of the enzyme was derived. Transcriptional analysis now indicates that the RNA start site is located 80 base pairs before the methionine codon initiating the open-reading frame. Further analysis of the DNA polymerase gene of a phosphonoacetate-resistant mutant virus revealed that a single nucleotide substitution changing glycine at amino acid 347 to aspartic acid results in drug resistance. This mutation may mark a part of the catalytic site of the enzyme.</p> <p>Studies on the mechanism of formation of the hairpin telomeres are continuing. Evidence has been obtained that the ends are formed by resolution of near perfect palindromes separating unit genomes in large concatemers. An in vivo transfection assay for the resolution reaction was developed and the minimal sequence requirement is being investigated.</p> <p>A search for origins of vaccinia DNA replication revealed that any plasmid that is transfected into cells infected with vaccinia virus is replicated. Although this phenomenon is specific for vaccinia virus infected cells, its significance is not understood.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00126-13 LVD
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Functional Analysis of Vaccinia Virus DNA		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: F. M. DeFilippes Research Physicist LVD, NIAID		
Others:		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Viral Diseases		
SECTION Macromolecular Biology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205		
TOTAL MAN-YEARS: 1.1	PROFESSIONAL: 1.0	OTHER: 0.1
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) A vaccinia virus mutant which is resistant to aphidicolin was shown to code for a drug-resistant DNA polymerase. Aphidicolin resistance was transferred to cells infected with wild-type virus by transfection with progressively smaller DNA segments derived from the DNA polymerase gene of the resistant virus. After a 124 bp segment which conferred resistance was cloned in M13 phage, the sequence of one viral strand was obtained and it showed that the mutant had a T replacing a parental G at a single site. Efforts to sequence the other strand by reversing the orientation of the segment were unsuccessful; however, a sequence of the complementary strand was derived from the plasmid containing the mutated segment and it had an A, replacing a C, at the correct site. Site-directed mutagenesis of the wild-type DNA should confer aphidicolin resistance. To facilitate this research, several techniques were developed. One used a chimeric phage-plasmid vector (phagemid), that has two replication origins which allows it to replicate as double-stranded or single-stranded DNA. Production of the latter which is used for sequencing and site-directed mutagenesis, depends on activating the fl origin of the phagemid by helper phage. To utilize phagemids for vaccinia DNA analysis conditions were established for selecting and growing the helper phage and bacteria containing the recombinant phagemids, and for maximizing the amount of single-stranded DNA which was secreted from bacteria. Another development improved the recovery of small DNA segments from agarose gels. A commercial electrophoresis chamber with a collection well where high salt solutions were supposed to trap electrophoretically transferred DNA, did not work well. Investigation of different solutions showed that the high buoyant density of a concentrated CsCl solution supplemented the effect of the high counter-ion concentration and caused the DNA to concentrate in the collection well. This procedure is probably the simplest and most efficient way to recover small to medium size DNA.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00298-05 LVD
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Development of Vaccinia Virus as an Expression Vector		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: B. Moss Laboratory Chief LVD, NIAID		
Others: K. Brechling Guest Researcher LVD, NIAID T. Fuerst Staff Fellow LVD, NIAID F. Falkner Guest Researcher LVD, NIAID C. Flexner Medical Staff Fellow LVD, NIAID		
COOPERATING UNITS (if any) CDC (J. Esposito); University of Washington (T. Yilma); LOM, NIDR (A.L. Notkins); Brookhaven National Laboratory (F.W. Studier); State University of New York (R.C. Condit).		
LAB/BRANCH Laboratory of Viral Diseases		
SECTION Macromolecular Biology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205		
TOTAL MAN-YEARS: 2.4	PROFESSIONAL: 2.15	OTHER: 0.35
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Procedures have been developed for the use of vaccinia virus as a eukaryotic expression vector. A chimeric gene is formed by ligating vaccinia virus transcriptional regulatory signals to a foreign protein coding sequence. Homologous recombination is used to insert the chimeric gene into a non-essential region of the vaccinia virus genome. To facilitate the formation and isolation of recombinant virus, new plasmid vectors have been constructed that have stronger promoters and multiple cloning sites and which direct the insertion of the chimeric gene together with the <i>E. coli</i> β-galactosidase gene into the thymidine kinase locus. Recombinant virus is then selected on the basis of the thymidine kinase negative phenotype and/or staining with a β-galactosidase indicator dye. The recombinant viruses produced in this manner are stable and have a wide host range for tissue culture cells and animals. This system has been used to express genes from a variety of infectious agents including herpes simplex virus type 1, hepatitis B virus, influenza virus, vesicular stomatitis virus and rabies virus. Animals vaccinated with each of the above recombinants were protected against challenge with the corresponding virus.</p> <p>A new eukaryotic transient expression system based on recombinant virus that synthesizes bacteriophage T7 RNA polymerase has been developed. This new system is about 500-fold more efficient than previous transient expression systems that rely on the enhancer and promoter elements of the long terminal repeat of Rous sarcoma virus or the early region of SV40.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00306-05 LVD
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Pathogenesis of Orthopoxvirus Infections		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: R.M.L. Buller Visiting Scientist LVD, NIAID		
Others: B. Moss Laboratory Chief LVD, NIAID G. Kotwal Visiting Associate LVD, NIAID A. Huggins Guest Researcher LVD, NIAID		
COOPERATING UNITS (if any) LIP, NIAID (H.C. Morse, III, R. Yetter and K. Holmes); University of Connecticut (T. Frederickson).		
LAB/BRANCH Laboratory of Viral Disease		
SECTION Macromolecular Biology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 1.9	PROFESSIONAL: 1.0	OTHER: 0.9
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>In this project we focus our studies on the genetic basis both of orthopoxvirus virulence and of host resistance to virus infection. The acquired knowledge should contribute towards the development of safe, effective, recombinant vaccinia virus vaccines for animal and human use. In this year, two separate approaches have been initiated to identify orthopoxvirus virulence genes. The first involves DNA sequence analysis of a 9 kb DNA stretch of vaccinia virus genome previously shown to be non-essential for replication in tissue culture, but important in virus virulence in the mouse. The second involves developing an insertional inactivation mutagenesis system suitable for screening large regions of the ectromelia virus genome for functions important in virus virulence. Using ectromelia virus and two separate experimental approaches, it was shown that recovery from a primary infection did not require the synthesis of anti-ectromelia virus neutralising antibody. These experiments also showed that a normal anti-ectromelia CTL response could be generated in the absence of L3T4⁺ ("helper" T-cells). This CTL maturation pathway may be of general importance in resistance to a variety of agents that replicate in cells and express antigens at the cell surface. Genetic analysis of crosses between susceptible (A/J) and resistant (C57BL/6J) mice indicated that at least two non-H2 genes were important in the recovery of mousepox. Studies are aimed at producing a mouse strain A.B6-Rmp (Resistance Mouse Pox).</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00391-03 LVD
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Expression of Genes of Respiratory Viruses by Vaccinia Virus Recombinants		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: N. Elango Visiting Associate LVD, NIAID Others: B. Moss Laboratory Chief LVD, NIAID		
COOPERATING UNITS (If any) University of Cambridge (G. L. Smith); The Wistar Institute (J. R. Bennink and J. W. Yewdell); LID, NIAID (B. Murphy, P. Collins, G. Prince, and R. Chanock); University of Oxford (A. McMichael).		
LAB/BRANCH Laboratory of Viral Diseases		
SECTION Macromolecular Biology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205		
TOTAL MAN-YEARS: 0.95	PROFESSIONAL: 0.6	OTHER: 0.35
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Development of effective vaccines against respiratory viruses requires a thorough understanding of the immunological response to individual proteins. We have chosen two viruses, influenza virus type A and respiratory syncytial (RS) viruses for detailed analysis. Individual recombinant vaccinia viruses that express the ten influenza virus genes have been constructed. Mice vaccinated with the vaccinia/influenza hemagglutinin recombinant made neutralizing antibodies and are protected against lower respiratory infection with influenza virus; however, the protection is subtype specific. Recombinants that express the nucleoprotein elicit a cross-reactive cytotoxic T cell response, but mice are not protected against infections.</p> <p>Evidence that humans also make cross-reactive cytotoxic T cells directed against the influenza nucleoprotein was demonstrated by infecting autologous human peripheral blood lymphoblastoid cells with recombinant vaccinia viruses.</p> <p>Recombinant vaccinia viruses that express the RS F and G proteins have been constructed. The proteins synthesized by the recombinants are glycosylated and transported to the plasma membrane. Cotton rats that are vaccinated with either recombinant produce neutralizing antibodies and are protected against lower respiratory infection with RS.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00392-03 LVD
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Expression of Hepatitis B Virus Genes by Vaccinia Virus Recombinants		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: K. Brechling Staff Fellow LVD, NIAID		
Others: K.-C. Cheng Guest Researcher LVD, NIAID B. Moss Laboratory Chief LVD, NIAID		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Viral Diseases		
SECTION Macromolecular Biology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205		
TOTAL MAN-YEARS: 0.75	PROFESSIONAL: 0.4	OTHER: 0.35
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) <p>Hepatitis is a serious world-wide health problem. Approximately 200 million people are chronically infected with hepatitis B virus, and large numbers of deaths are attributed to fulminant hepatitis, cirrhosis, and hepatocellular carcinoma. Although an effective subunit vaccine has been produced, limitations in supply and expense have prevented its global use. As an alternative, we are trying to construct a live recombinant hepatitis B vaccine. The gene for hepatitis B virus surface antigen has been engineered and inserted into the genome of vaccinia virus. The recombinant vaccinia virus is stable and expresses the hepatitis virus protein. The latter is glycosylated, assembled into particles and transported through the plasma membrane of infected cells. Rabbits vaccinated with the recombinant virus produce a high and sustained specific antibody response. Vaccination of chimpanzees resulted in priming of the immune system and protection against clinical hepatitis upon subsequent challenge with hepatitis B virus. Vaccinia virus recombinants that express higher levels of HBsAg have been constructed by using the promoter from a major structural protein of vaccinia virus and are being evaluated.</p> <p>Recent studies have indicated that the DNA sequence preceding the HBsAg gene, referred to as pre-S, is expressed by hepatitis B virus and contains immunologically dominant epitopes. A vaccinia virus recombinant that expresses the entire long open-reading-frame and produces the large surface protein has been constructed. Another recombinant virus that contains a part of the pre-S region and synthesizes the middle surface protein also was made. Both recombinant vaccinia viruses induce antibodies to pre-S and S epitopes when inoculated into rabbits.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00393-03 LVD
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Expression of Malaria Genes by Vaccinia Virus Recombinants		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: B. Moss Laboratory Chief LVD, NIAID		
Others: K.-C. Cheng Guest Researcher LVD, NIAID		
COOPERATING UNITS (if any) New York University (R. and V. Nussenzweig); Walter and Eliza Hall Institute (C. Langford); LPD, NIAID (L. Miller); University of Cambridge (G. L. Smith)		
LAB/BRANCH Laboratory of Viral Diseases		
SECTION Macromolecular Biology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205		
TOTAL MAN-YEARS: 0.45	PROFESSIONAL: 0.1	OTHER: 0.35
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Malaria remains a serious global health problem for which there is no effective vaccine. Previous studies indicate that animals can be immunized with inactivated sporozoites. The gene coding for the circumsporozoite (CSP) antigen of the malaria parasite <u>Plasmodium falciparum</u> was inserted into the vaccinia virus genome under the control of a defined vaccinia virus promoter. Tissue culture cells infected with the recombinant synthesized polypeptides that reacted with monoclonal antibody against the malaria protein. Studies on the sequence of the expressed <u>P.falciparum</u> CSP indicated that the NH₂-terminus is blocked and COOH-terminus is not processed. Immunofluorescent staining demonstrated that the CSP was distributed primarily in the cytoplasm of infected cells. Mice and rabbits vaccinated with the recombinant virus produced antibodies that bound specifically to sporozoites.</p> <p>The S-antigen gene of <u>P. falciparum</u> also was expressed in a vaccinia virus recombinant. The protein was secreted from infected cells and reacted with specific antiserum. Vaccinated animals produced a low but detectable antibody response. By attaching the transmembrane domain of an immunoglobulin gene to the S-antigen gene, a new form of the plasmodial protein was synthesized. This chimeric protein was no longer secreted but became attached to the plasma membrane. Significantly, rabbits immunized with the new recombinant vaccinia virus developed much higher antibody levels to S-antigens.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00416-03 LVD
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Recombinant Vaccines Against Retroviruses Associated with Leukemia and AIDS		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: S. Chakrabarti Visiting Associate LVD, NIAID		
Others: P. Earl Senior Staff Fellow LVD, NIAID F. Falkner Guest Researcher LVD, NIAID B. Moss Laboratory Chief LVD, NIAID		
COOPERATING UNITS (if any) LPVD, NIAID (B. Chesebro); LTCB, NCI (F. Wong-Staal and R. Gallo), University of Alabama (R. W. Compans)		
LAB/BRANCH Laboratory of Viral Diseases		
SECTION Macromolecular Biology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205		
TOTAL MAN-YEARS: 2.05	PROFESSIONAL: 1.65	OTHER: 0.40
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> Retroviruses, long associated with leukemia and sarcoma of animals, have recently been implicated as the etiological agents of human T cell leukemia and human acquired immune deficiency syndrome (AIDS). The identification of these agents makes it possible to consider various ways of prevention. The most promising approach is development of a vaccine that could be administered to individuals at risk. Friend leukemia virus complex is a useful model system, since it produces an acute disease in adult mice which can be prevented by repeated immunization with the envelope glycoprotein. The envelope (env) gene from Friend murine leukemia virus (F-MuLV) was inserted into the genome of a vaccinia virus expression vector. Infected cells synthesized gp85, the glycosylated primary product of the env gene. Processing to gp70 and p15E, and cell surface localization, were similar to that occurring in F-MuLV infected cells. Mice that were inoculated with the live recombinant vaccinia virus had an envelope-specific T cell proliferative response and following challenge with FV complex developed neutralizing antibody and cytotoxic T cells (CTL) and were protected against leukemia. In contrast, unimmunized and control groups developed a delayed neutralizing antibody response but no detectable CTL and succumbed to leukemia. Genes of the major histocompatibility complex (H-2) influenced protection induced by the vaccinia recombinant but not that induced by attenuated N-tropic Friend virus. A similar approach was used to insert the env gene of the AIDS virus into the genome of vaccinia virus. Formation of the gp160 precursor and processing to gp120 and gp41 occurred as in cells infected with AIDS virus. Furthermore, mice inoculated with the recombinant virus developed antibodies to gp120. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00443-02 LVD
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Vaccinia Virus Growth Factor		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: R. M. L. Buller Visiting Scientist LVD, NIAID		
Others: B. Moss Laboratory Chief LVD, NIAID S. Chakrabarti Visiting Associate LVD, NIAID		
COOPERATING UNITS (if any) ONCOGEN (D. Twardzik); Fred Hutchinson Research Laboratories (J. Cooper); Microgenysis (M. Cochran).		
LAB/BRANCH Laboratory of Viral Diseases		
SECTION Macromolecular Biology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205		
TOTAL MAN-YEARS: 0.4	PROFESSIONAL: 0.3	OTHER: 0.1
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> Vaccinia virus has two copies of a gene which has a 50-amino acid predicted sequence homology with the receptor binding site of epidermal growth factor (EGF) and transforming growth factor (TGF-α). A 25 kilodalton glycoprotein (vaccinia virus growth factor [VGF] that can bind to EGF receptors) has been purified from media of virus infected BS-C-1 cells. When a mutant virus (VGF⁻) was constructed by removing both copies of the sequence encoding the putative EGF receptor binding site, the EGF receptor binding activity in infected cells was abolished. Inoculations of the VGF⁻ mutant into BALB/cByJ mice by the intracranial route resulted in an LD₅₀ which was 100-fold higher than the wild-type (WT) virus. A similar attenuated phenotype was observed with the mutant on intradermal inoculation of the skin of the New Zealand white rabbit. The growth of this mutant was extensively investigated in BSC-1 and A431 (high density of EGF receptors on cells) cell lines, and on the chorio-allantoic membrane (CAM) of the chicken egg. BS-C-1 cells maintained either in high or low fetal calf serum supported the replication of the VGF⁻ mutant to the same degree as WT. In A431 cells, the mutant produced a similar yield of progeny virus as WT in the growth conditions examined, although the plaque morphology of the mutant was markedly different from the WT. The WT virus showed a piling up of cells around the foci of infection. The mutant, on the other hand, produced a typical plaque indistinguishable from that observed on BS-C-1 cells. On the CAM of the egg, both WT and mutant virus formed pocks with approximately the same efficiency. The WT, but not the mutant, induced extensive ectoderm and endoderm proliferation. WT lesions were on the whole slightly larger than the mutant, and contained greater amounts of infectivity. Both viruses induce a similar inflammatory response in the host which was observed at 40 hours post inoculation. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00444-02 LVD
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Regulation of Expression of Herpes Simplex Virus Genes		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: J. P. Weir Senior Staff Fellow LVD, NIAID		
Others: P. R. Narayanan Guest Worker LVD, NIAID		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Viral Diseases		
SECTION Macromolecular Biology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205		
TOTAL MAN-YEARS: 1.7	PROFESSIONAL: 1.7	OTHER:
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues x <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The expression of the HSV-1 glycoprotein C (gC) gene was studied by using a chimeric gene that was constructed by cloning the promoter sequences of the gC gene upstream of the coding sequences for the bacterial enzyme β-galactosidase (β-gal), such that expression of β-gal was directed by the gC regulatory region. This chimeric gene was cloned as a unit into the middle of the coding sequences of the HSV-1 thymidine kinase (TK) gene and, by homologous recombination, was inserted into the HSV-1 genome at the TK locus. A recombinant virus containing the chimeric gene was isolated by screening for TK⁻ virus that expressed β-gal, and this virus was shown to express β-gal as a late HSV-1 gene. To define the sequences necessary for viral late gene regulation, a series of deletions was made from the 5' end of the 1450 base-pair gC regulatory region and the effect of the deletions on β-gal expression was examined both in transient assays and in recombinant viruses. In both types of assay, deletions which extended to -104 bp above the mRNA start site had no effect on β-gal expression, indicating that late gene regulatory signals in the 5' region of the gC gene lie within this 104 bp. Other deletions have been made in both the 5' and the 3' regions of the gC regulatory sequences and are being used to further define the elements of regulatory control for the gC gene. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00445-02 LVD
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Mechanisms of Viral DNA Replication		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Mark D. Challberg Senior Staff Fellow		
Others: Carol Wu Staff Fellow Paul Olivo Medical Fellow		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Viral Diseases		
SECTION Macromolecular Biology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205		
TOTAL MAN-YEARS: 3.35	PROFESSIONAL: 2.25	OTHER: 1.1
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> Herpes simplex virus is a useful model for studying the mechanisms involved in DNA replication in eukaryotic cells. Our current efforts are directed toward studying these processes with purified proteins. To this end, we have developed a transfection assay which has allowed us to identify all of the viral genes necessary for viral DNA replication. This system is based on results from several laboratories showing that transfected plasmid DNAs containing either of the two known origins of DNA replication, ORI_S and Ori_L, are replicated in HSV-1 infected cells. We have found that a combination of five cloned restriction fragments of HSV-1 can supply all of the functions necessary for the replication of plasmids containing an HSV origin of replication. These five fragments are: XbaI C (0.0 - 0.294), XbaI F (0.294 - 0.453), XbaI E (0.453 - 0.641), XbaI D (0.641 - 0.830), and EcoRI JK (0.0 - 0.086; 0.830 - 0.865). We have now sub-cloned each of these fragments to precisely locate the essential genes, and have found a total of eight essential genes. This information will be used to purify each of the viral gene products involved in DNA replication, and the purified proteins will be used as a starting point to reconstruct DNA replication <u>in vitro</u>. </p>		

11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
66
67
68
69
70
71
72
73
74
75
76
77
78
79
80
81
82
83
84
85
86
87
88
89
90
91
92
93
94
95
96
97
98
99
100



LABORATORY OF MICROBIAL STRUCTURE AND FUNCTION
Rocky Mountain Laboratories
Hamilton, Montana
1986 Annual Report
Table of Contents

<u>Z01 AI</u> <u>Project Number</u>		<u>Page</u>
Summary		13-1
00065-13	Antigens and Classification of Rickettsiae -- Anacker	13-7
00193-07	Gonococcal Surface Components: Structure and Function -- Swanson	13-8
00216-06	Immunology of Chlamydial Surface Antigens -- Caldwell	13-9
00234-05	Biology of Intracellular Parasitism -- Hackstadt	13-10
00235-05	Studies on Nonprotein Surface Constituents of Three Venereal Bacteria -- Hitchcock	13-11
00412-03	Molecular Analysis of the Lyme Disease Spirochete -- Howe (terminated)	13-12
00413-03	Molecular Genetics of <u>Chlamydia trachomatis</u> -- Nano	13-13
00441-02	Cloning and Expression of Genes of <u>Rickettsia rickettsii</u> in <u>Escherichia coli</u> -- McDonald	13-14
00442-02	Immunobiology of Guinea Pig Inclusion Conjunctivitis - Watkins	13-15

Annual Report
Laboratory of Microbial Structure and Function
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1985, to September 30, 1986

RESEARCH HIGHLIGHTS

Major emphasis in LMSF is on definition of structural and functional elements of pathogenic bacterial surface components involved in pathogenesis and/or virulence of selected organisms or in genesis of hosts' immunological responses to infections by these agents. Investigative attention in LMSF is directed toward both extracellular pathogens (including Neisseria gonorrhoeae, Contagious Equine Metritis Organism, and Borrelia burgdorferi) and obligate intracellular parasites (including Chlamydia trachomatis, Chlamydia psittaci, Rickettsia rickettsii, and Coxiella burnetii). Both protein and nonprotein components of these gram-negative organisms' outer membrane are prime study candidates as mediators of interactions between bacterium and host and as likely vaccine components. Chemical characteristics, immunochemical properties, and genetic control of selected surface components are investigated to delineate their relationship to infectious disease phenomena of these bacteria. A number of findings emanating from studies in LMSF during the past year are summarized below:

Chlamydiae: Previous findings on chlamydial major outer membrane protein (MOMP) and lipopolysaccharide (LPS) by LMSF scientists were extended and embellished by utilization of experimental animal models (guinea pig and cynomolgus monkeys) of primary conjunctival infection for assessment of immunity and hypersensitivity generated by such infections. Following the spontaneous resolution of primary chlamydial infections of their conjunctivae, guinea pigs and monkeys are immune to reinfection by chlamydiae of homologous serotype; this immunity correlates with production of lacrimal IgA antibodies directed against MOMP. But these immuno-protected animals also develop a pronounced ocular hypersensitivity to chlamydial antigens which can be extracted from viable organisms with Triton X-100. Primary chlamydial infections at vaginal or intestinal loci also render guinea pigs hypersensitive to conjunctival challenge with either large numbers of viable chlamydiae or their Triton-soluble components. Repeated conjunctival application of these chlamydial extracts produces recurring ocular reactions that mimic trachoma clinically and histopathologically. This ocular hypersensitivity undoubtedly contributes to development of the delayed, chronic trachomagenic sequelae of mucosal infections by chlamydiae. These findings add considerable new information related to the pathogenesis of trachoma in geographical areas where chlamydial conjunctivitis and trachoma are endemic, and they should be highly useful for future evaluation of vaccines designed to prevent or ameliorate the delayed sequelae of chlamydial infections. These studies of guinea pig and monkey models have been conducted by Harlan Caldwell in collaboration with Nancy Watkins (LMSF) and Hugh Taylor (Johns Hopkins), respectively. Monoclonal antibodies directed toward several different epitopes of MOMP have been produced; passive protection against lethal injections of homologous chlamydiae is afforded mice by monoclonal antibodies directed toward heat-sensitive epitopes that are accessible on the chlamydial surface. Other anti-MOMP monoclonals directed against inaccessible or heat-stable, accessible epitopes as well as anti-LPS monoclonals do not display such protection. These studies have been executed by Caldwell and Y-X. Zhang; they provide new information relevant to development of a protective vaccine against chlamydial infections. Fran Nano has cloned genes encoding a glycosyl transferase involved in chlamydial LPS synthesis; these recombinant molecules function in a broad range of enterobacteria including the various LPS

chemotypes of salmonellae in which the LPS products have been chemically analyzed to pinpoint the influence of their possessing the chlamydial enzyme; these analytical studies are being pursued by Nano in collaboration with Helmut Brade (Borstel, FRD) and provide important insight into the bacterial synthesis of LPS. Nano has also cloned chlamydial DNA encoding MOMP in collaboration with Theresa Joseph (LMSF); the cloned MOMP gene will provide a badly needed reagent for assessing biological and immunological reactivities of MOMP. Ted Hackstadt has identified two proteins on chlamydial elementary bodies that bind HeLa cell surface components; these chlamydial proteins may function as adhesins for initial attachment to host cells by these obligate intracellular pathogens. Heparin desorbs attached chlamydia from HeLa cell surfaces and also inhibits binding of HeLa cell surface components to the chlamydial adhesin proteins. A monoclonal antibody reactive with these adhesin proteins has been obtained (from J. Newhall, Univ. Indiana) and exhibits binding also to HeLa cells. These developments have interesting implications relating to lack of immunogenicity observed for these chlamydia adhesin proteins.

Rickettsiae: Bob Anacker has defined several monoclonal antibodies that passively-protect mice against lethal challenge with viable R. rickettsii. One such monoclonal recognizes an epitope on a 155-kD protein whose encoding gene has been molecularly cloned into E. coli by Greg McDonald; E. coli containing the recombinant plasmid were used as a vaccine which is capable of eliciting protection for mice and guinea pigs challenged with viable R. rickettsii. This recombinant will be used for further definition and characterization of its 155-kD polypeptide product and for its evaluating its utility as an effective vaccine against Rocky Mountain spotted fever. Monoclonal reagents that recognize LPS on intact rickettsiae are not protective. LPS variation in Coxiella burnetii has been documented by Hackstadt and immunological and biological relevance of such LPS variants is being investigated by Abbie Moos in collaboration with Hackstadt; C. burnetii isolates from endocarditis cases exhibit a distinct LPS, but immunization with that LPS engendered cross-protection against fever-causing capacities of C. burnetii of both homologous and heterologous LPS-bearing organisms.

Gonococci: Genetic control of pilus production by gonococci was examined by John Swanson in collaboration with Sven Bergstrom (LMSF) and Michael Koomey (Rockefeller Univ.). They demonstrated that mutational changes in the pilin structural gene can account for production of pilin subunits that are not assembled into pili and, hence, yield a pilus⁻ phenotype. In recombination-deficient gonococci, these mutations represent spontaneous addition/deletion/substitution changes in the pilin structural gene. In wild type gonococci, gene conversion of the pilin gene by new "cassette" of pilin-encoding sequence may result in synthesis of either a novel but "orthodox" pilus subunit and pilus formation or of an "unorthodox" pilin subunit from which pili are not assembled; so the former have pilus⁺ phenotype while the latter are pilus⁻. Swanson also found, in collaboration with John Boslego (WRAIR), that gonococci inoculated into the human male urethra underwent gene conversion of their pilin structural gene and elaborated a different pilus subunit than that made by gonococci comprising the inoculum. This is the first time such a change has been found in the context of a human gonorrheal infection. Also demonstrable was a marked change in the expression of outer membrane protein II in gonococci isolated versus inoculated in the human volunteer study. Penny Hitchcock and Wolfgang Strittmatter explored several biological and biochemical parameters of the gonococcal surface antigen H.8. This antigen has a polypeptide portion exhibiting unusual amino acid composition and also contains two fatty acid constituents. Although H.8 antigens

from all studied gonococci exhibit a common epitope (by monoclonal antibody reactivity), the apparent subunit sizes and surface-accessibilities of H.8 molecules may vary among different gonococci. Surface-accessibility of the H.8 epitope correlated with liability of gonococci to bactericidal action of anti-H.8 monoclonal antibody, and may also relate to resistance/susceptibility of gonococci to killing by normal human serum. Hitchcock also continued her studies on the elusive capsule of gonococci including preliminary biochemical and antigenic analyses.

Annual Report
Laboratory of Microbial Structure and Function
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1985, to September 30, 1986

ADMINISTRATIVE REPORT

Personnel changes during the past year include arrival of one senior staff fellow, Dr. Wolfgang Baehr; one visiting associate, Dr. Suryanarayanan Vishwanath; and one visiting fellow, Ms. Hua Su; and departure of Dr. Timothy Howe (to Hybritech, Inc., San Diego, CA); Dr. Sven Bergström (to Umea University, Umea, Sweden); and Dr. Robert Anacker (retirement). Two members of LMSF technical staff departed (Robert List, retirement, and Lisa Milch, to medical school). Seminars were given by visitors as follows: Patrick Brennan, University of Colorado School of Medicine, Fort Collins); Bianca Colonna (University of Rome, Italy); Tom Dougherty (Rockefeller University, New York, NY); Sam Formal (Walter Reed Army Medical Center, Washington, DC); Ake Hagstrom (University of Umea, Umea, Sweden); Tom Hatch (University of Tennessee, Memphis); Ned Hook (Johns Hopkins University, Baltimore, MD); Harry Jennings (National Research Council, Ottawa, Canada); Alf Lindberg (Karolinska Institute, Huddinge, Sweden); Richard Losick (Harvard University, Cambridge, MA); Mike Parmely (University of Kansas, Kansas City); Eric Milner (University of Texas Southwest Medical Center, Dallas); Ward Robinson, University of Utah School of Medicine, Salt Lake City); Abigail Salyers (University of Illinois, Urbana); Jim Samuels (Washington State University, Pullman); Ken Sanderson (University of Calgary, Calgary, Canada); Pam Small (Stanford University, Stanford, CA); and Bruce Stocker (Stanford University, Stanford, CA). The Summer Seminar Session, organized by John Swanson, was held at the Rocky Mountain Laboratories, July 7-9, with 18 participants from Rockefeller University (Laboratory of Bacteriology, Emil Gotschlich and colleagues), Stanford Medical School (Department of Medical Microbiology, Stanley Falkow's group), and Harvard Medical School (John Mekalanos, special invited guest); 16 staff members of LMSF and LPB participated.

from all studied gonococci exhibit a common epitope (by monoclonal antibody reactivity), the apparent subunit sizes and surface-accessibilities of H.8 molecules may vary among different gonococci. Surface-accessibility of the H.8 epitope correlated with liability of gonococci to bactericidal action of anti-H.8 monoclonal antibody, and may also relate to resistance/susceptibility of gonococci to killing by normal human serum. Hitchcock also continued her studies on the elusive capsule of gonococci including preliminary biochemical and antigenic analyses.

Annual Report
Laboratory of Microbial Structure and Function
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1985, to September 30, 1986

ADMINISTRATIVE REPORT

Personnel changes during the past year include arrival of one senior staff fellow, Dr. Wolfgang Baehr; one visiting associate, Dr. Suryanarayanan Vishwanath; and one visiting fellow, Ms. Hua Su; and departure of Dr. Timothy Howe (to Hybritech, Inc., San Diego, CA); Dr. Sven Bergström (to Umea University, Umea, Sweden); and Dr. Robert Anacker (retirement). Two members of LMSF technical staff departed (Robert List, retirement, and Lisa Milch, to medical school). Seminars were given by visitors as follows: Patrick Brennan, University of Colorado School of Medicine, Fort Collins); Bianca Colonna (University of Rome, Italy); Tom Dougherty (Rockefeller University, New York, NY); Sam Formal (Walter Reed Army Medical Center, Washington, DC); Ake Hagstrom (University of Umea, Umea, Sweden); Tom Hatch (University of Tennessee, Memphis); Ned Hook (Johns Hopkins University, Baltimore, MD); Harry Jennings (National Research Council, Ottawa, Canada); Alf Lindberg (Karolinska Institute, Huddinge, Sweden); Richard Losick (Harvard University, Cambridge, MA); Mike Parmely (University of Kansas, Kansas City); Eric Milner (University of Texas Southwest Medical Center, Dallas); Ward Robinson, University of Utah School of Medicine, Salt Lake City); Abigail Salyers (University of Illinois, Urbana); Jim Samuels (Washington State University, Pullman); Ken Sanderson (University of Calgary, Calgary, Canada); Pam Small (Stanford University, Stanford, CA); and Bruce Stocker (Stanford University, Stanford, CA). The Summer Seminar Session, organized by John Swanson, was held at the Rocky Mountain Laboratories, July 7-9, with 18 participants from Rockefeller University (Laboratory of Bacteriology, Emil Gotschlich and colleagues), Stanford Medical School (Department of Medical Microbiology, Stanley Falkow's group), and Harvard Medical School (John Mekalanos, special invited guest); 16 staff members of LMSF and LPB participated.

Annual Report
Laboratory of Microbial Structure and Function
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1985, to September 30, 1986

HONORS AND AWARDS

Journal Editorial Boards:

J. Swanson - Infection and Immunity
P. Hitchcock - Journal Bacteriology

Manuscripts were reviewed by LMSF staff for the following journals:
Canadian Journal of Microbiology, Infection and Immunity, Journal of Bacteriology,
Journal of Biological Chemistry, Journal of Clinical Investigation, Journal of
Clinical Microbiology, Journal of General Microbiology, Journal of Immunology,
Journal of Infectious Diseases, Proceedings of the National Academy of Sciences,
USA, Science, and Sexually Transmitted Diseases.

Professional Posts:

J. Swanson - Member, Microbiology and Infectious Diseases Research Committee,
NIAID
O. Barrera - Promotion Advisory Committee, NIAID
H. Caldwell - Faculty Affiliate, Department of Microbiology, University of
Montana, Missoula, MT
Promotion Advisory Committee, NIAID
T. Hackstadt - Consultant Member, Food and Drug Administration, Orphan Products
Development Initial Review Group, Rockville, MD

Invited Lectures and Participation in Meetings and Symposia:

J. Swanson - Divisional Lecturer, American Society for Microbiology, Washington, DC
International Conference on Pathogenic Neisseria, Amsterdam,
The Netherlands
H. Caldwell - Sixth International Symposium on Human Chlamydial Infections,
Surrey, England
North Carolina State held at Research Triangle Park, NC
T. Hackstadt - Sixth International Symposium on Human Chlamydial Infections,
Surrey, England
UA-UC Conference on Infectious Diseases, Invermere, BC, Canada
Max-Planck-Institut für Immunbiologie, Frieberg, Germany
University of California at Davis, CA
University of Wisconsin, Madison, WI

P. Hitchcock - Agouron Institute, La Jolla, CA
University of Illinois School of Medicine, Chicago, IL
University of North Carolina, Chapel Hill, NC
International Conference on Pathogenic Neisseria, Amsterdam,
The Netherlands

F. Nano - Sixth International Symposium on Human Chlamydial Infections,
Surrey, England
University of Umea, Umea, Sweden
Institut fur Experimentelle Biologie und Medizin, Borstel, Germany

W. Strittmatter - Rockefeller University, New York, NY

N. Watkins - Sixth International Symposium on Human Chlamydial Infections,
Surrey, England
University of South Carolina School of Medicine, Columbia, SC

Y-X. Zhang - Sixth International Symposium on Human Chlamydial Infections,
Surrey, England

Other Activities:

H. Caldwell - Reviewed research grants for British Columbia Health Care Research
Foundation, British Columbia, Canada, and The Edna McConnell
Clark Foundation, New York, NY

T. Hackstadt - Reviewed research grants for British Columbia Health Care Research
Foundation, British Columbia, Canada

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00065-13 LMSF
PERIOD COVERED October 1, 1985, to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Antigens and Classification of Rickettsiae		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: R. L. Anacker Research Microbiologist LMSF, NIAID Others: G. A. McDonald Staff Fellow LMSF, NIAID		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Microbial Structure and Function, Hamilton, MT 59840		
SECTION		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20205		
TOTAL MAN-YEARS: <div style="text-align: center;">2.75</div>	PROFESSIONAL: <div style="text-align: center;">1.0</div>	OTHER: <div style="text-align: center;">1.75</div>
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> Efforts have been continued to identify and characterize the constituents responsible for the biological activities of the etiologic agent of Rocky Mountain spotted fever, <u>Rickettsia rickettsii</u>. Mice immunized with a soluble rickettsial fraction, purified by affinity chromatography and containing principally the 120-kd surface protein but also detectable amounts of several other components, produced two kinds of antibodies: one specific for RP 120 and one specific for the LPS-like antigen. Since antibodies to the LPS-like antigen do not protect mice, it seems probable that RP 120 was responsible for the immunity developed in mice. RP 120 was found to be hydrophobic in a detergent phase separation experiment; the hydrophobic properties of this protein may account for our difficulties in purifying RP 120. Guinea pigs inoculated with monoclonal antibodies specific for RP 120 or RP 155 were partially protected against challenge with <u>R. rickettsii</u>. Antibodies to the LPS-like antigen did not protect. Four kinds of monoclonal antibodies were derived from mice inoculated with rickettsiae inactivated at 56°C for 15 min. Three kinds were identical to those obtained from mice given viable rickettsiae; the newly-observed kind recognized heat-resistant (HR) epitopes on RP 155. Antibodies to the HR epitopes did not protect mice from lethal challenge with <u>R. rickettsii</u>. Epitopes recognized by our monoclonal antibodies are distributed widely among spotted fever group rickettsiae; these antibodies could be used to identify all of the species of the group, as well as to differentiate strains within at least one species. In addition, one of the monoclonal antibodies to a HR epitope of RP 155 reacted strongly with the corresponding protein of <u>R. canada</u>, a member of the typhus group. Monoclonal antibodies to the LPS-like antigen reacted with all members of the spotted fever group by ELISA. This antigen probably is the group antigen shared by all members of the group. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00193-07 LMSF
PERIOD COVERED October 1, 1985, to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Gonococcal Surface Components: Structure and Function		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: J. Swanson Chief LMSF, NIAID		
Other: S. Bergstrom Visiting Fellow LMSF, NIAID O. Barrera Microbiologist LMSF, NIAID K. Robbins Microbiologist LMSF, NIAID D. Corwin Bio. Lab. Tech. LPB, NIAID		
COOPERATING UNITS (if any) Michael Koomey, Rockefeller University, New York, NY John Boslego, WRAIR, Washington, DC		
LAB/BRANCH Laboratory of Microbial Structure and Function, Hamilton, MT 59840		
SECTION		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20205		
TOTAL MAN-YEARS 3.85	PROFESSIONAL: 1.5	OTHER: 2.35
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> Gonococci (Gc) are thought to require pilus⁺ phenotype for virulence, but pilus⁻ status may also confer advantages on Gc in transmission to other hosts, spread in an individual, etc., if these organisms are capable of reversion to pilus⁺. In vitro, Gc display pilus⁺ pilus⁻ "phase variation" at high frequency; at similarly high frequency, pilus⁺ Gc also spawn variant pilus⁻ progeny that express structurally/antigenically distinct pili. Our current studies focus on the molecular mechanisms responsible for both phase and pilus structural changes. Work during the present year has documented gene conversion as accounting for both these changes in piliation. This occurs via non-reciprocal homologous recombination involving the Gc genome's single complete, expressed pilin structural gene and one of the multiple, silent, storage partial pilin genes in the Gc chromosome. Whether pilus⁺ or pilus⁻ phenotype obtains correlates with primary structure of the pilin polypeptide encoded by the newly constituted "chimeric" pilin gene resulting from the gene conversion event and whether that pilin polypeptide can or cannot form mature, polymeric pili. Other studies on recombination-deficient (rec⁻) Gc have defined spontaneous mutation (single nucleotide deletion/insertion/substitution) in the pilin structural gene as accounting for changes in piliation status. Among the mutants isolated is one in which mutational change in the last amino acid in the pilin polypeptide's "leader" segment renders the pilin nonfunctional regarding pilus formation. Other piliation mutants in the rec⁻ Gc include "ochre" mutants synthesizing a truncated pilin polypeptide. Examination of Gc emanating from a experimental human infection has documented changes in both pilin structure and in protein II constitutions of Gc isolated versus Gc inoculated. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00216-06 LMSF
PERIOD COVERED October 1, 1985, to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Immunology of Chlamydial Surface Antigens		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: H. D. Caldwell Research Microbiologist LMSF, NIAID Others: F. E. Nano Staff Fellow LMSF, NIAID Y.-X. Zhang Visiting Fellow LMSF, NIAID		
COOPERATING UNITS (if any) Hugh Taylor, Johns Hopkins University School of Medicine, Baltimore, MD		
LAB/BRANCH Laboratory of Microbial Structure and Function, Hamilton, MT 59840		
SECTION Chlamydial Diseases Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20205		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.85	2.05	1.8
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Ocular chlamydial infections were studied in cynomolgus monkeys in order to evaluate the role of surface antigens in (i) resistance to reinfection and (ii) immunopathogenesis of disease.</p> <p>(i) <u>Resistance to reinfection.</u> Cynomolgus monkeys (<u>Macaca fascicularis</u>) were infected with a <u>C. trachomatis</u> trachoma biovar producing an acute follicular conjunctivitis. The temporal appearance of tear and serum antibodies reactive with <u>C. trachomatis</u> surface antigens were determined by immunoblotting analysis and radioimmunoprecipitation during this self-limiting disease. The chlamydial major outer membrane protein (MOMP) was found to be the primary immunogen recognized by monkey tear IgA antibodies during infection. The tear IgA antibody MOMP response was specific for the infecting trachoma serovar suggesting that antibodies directed against antigenically unique portions of the MOMP were protective. Monoclonal antibodies were generated against the MOMP that recognized antigenically unique epitopes and were found to neutralize the <i>in vivo</i> toxicity of the organism for mice and infectivity for the monkey eye. Purified native MOMP and recombinant MOMP are currently being tested as a subunit vaccine in the monkey model.</p> <p>(ii) <u>Immunopathogenesis of disease.</u> When administered onto the conjunctivae of immune but not naive monkeys, a Triton X-100 extract of chlamydiae produced an inflammatory response indistinguishable from that observed in monkeys with primary chlamydial conjunctivitis. This inflammatory response was not due to chlamydial infection and was characteristic of a delayed-type hypersensitivity reaction. The antigenic fraction that induced this deleterious immune response did not contain the protective MOMP antigen. Studies are currently underway aimed at characterization of this antigen. Preliminary results show that the deleterious antigen is heat labile and common to the genus chlamydiae.</p> <p>The significance of this project lies in the understanding of the pathogenesis of chlamydial diseases and in the development of a subunit chlamydial vaccine.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00234-05 LMSF
PERIOD COVERED October 1, 1985, to September 30, 1986		
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.) Biology of Intracellular Parasitism		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: T. Hackstadt Senior Staff Fellow LMSF, NIAID Others: A. B. Moos Staff Fellow LMSF, NIAID F. Nano Staff Fellow LMSF, NIAID		
COOPERATING UNITS (if any) Hubert Mayer, Max-Planck Institut, Freiberg, Jim Newhall, University of Indiana		
LAB/BRANCH Laboratory of Microbial Structure and Function, Hamilton, MT 59840		
SECTION		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20205		
TOTAL MAN-YEARS <div style="text-align: center;">3.0</div>	PROFESSIONAL: <div style="text-align: center;">1.75</div>	OTHER: <div style="text-align: center;">1.25</div>
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> This project examines mechanisms of pathogenesis of obligately intracellular bacteria. Primary emphasis is on the identification of surface structures which may function in host-parasite interactions, such as attachment or penetration, or otherwise act as determinants of virulence. Two obligately intracellular parasites with quite different modes of intracellular survival are presently being studied. <i>Chlamydia trachomatis</i>, the leading cause of preventable blindness worldwide and of sexually transmitted disease in this country, is being examined with a goal of identifying components which may function in the initial, adsorptive step of chlamydia-host interaction. Two surface proteins have been identified which display a number of properties that, collectively, suggest a role as adhesins. These proteins are present only on the infectious stage of the life cycle, display affinity for eucaryotic cell surface components, and vary among serotypes of <i>C. trachomatis</i> in correlation with the type of disease associated with a particular serotype. Studies of <i>Coxiella burnetii</i>, the etiological agent of Q fever, have focused on the lipopolysaccharides (LPS) since this component seems to be the predominant surface structure that varies in virulent to avirulent transitions. To explore the role of LPS in chronic human infections, we examined the LPS type from a number of <i>C. burnetii</i> strains. Three LPS groups, based either on SDS-PAGE profile or antigenic analysis, were identified. Two of these groups were comprised almost exclusively of isolates from Q fever endocarditis patients. This is the first demonstration of variation in the phase I antigen (LPS) of <i>C. burnetii</i>. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZOI AI 00235-05 LMSF
PERIOD COVERED October 1, 1985, to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Studies on Nonprotein Surface Constituents of Three Venereal Bacteria		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: P. J. Hitchcock Senior Staff Fellow LMSF, NIAID		
Others: W. G. Strittmatter Visiting Fellow LMSF, NIAID F. E. Nano Staff Fellow LMSF, NIAID T. M. Brown Microbiologist LMSF, NIAID M. D. Corwin Bio. Lab. Tech. LPB, NIAID S. F. Hayes Bio. Lab. Tech. LPB, NIAID		
COOPERATING UNITS (if any) John Boslego, WRAIR; Janne G. Cannon, U of NC; Emil Gotschlich, Rockefeller; Keith Joiner, LCI, NIAID; Alf Lindberg, Karolinska Institute, Huddinge, Sweden; Mark Peppler, U of Edmonton; E. N. Robinson, U of Utah		
LAB/BRANCH Laboratory of Microbial Structure and Function, Hamilton, MT 59840		
SECTION		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20205		
TOTAL MAN-YEARS: 3.45	PROFESSIONAL: 2.0	OTHER: 1.45
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The surface of gram-negative bacteria, the outer membrane, is a mosaic of tightly associated lipopolysaccharide (LPS) and protein molecules. In some organisms, the outer membrane itself is covered by loosely bound polymers which comprise the bacterial capsule. It is the surface of the bacterium which interacts with the host in early stages of parasitism, and it is the host defenses directed towards surface constituents which play a role in the prevention, resolution or sometimes exacerbation of bacterial diseases. We are engaged in the study of the structure and function of nonprotein surface constituents of <u>Neisseria gonorrhoeae</u> and to a minor degree those of <u>Chlamydia trachomatis</u> and <u>Tylorella equigenitalis</u> (Contagious Equine Metritis Organism, CEMO). Those neisserial constituents of particular interest are the H8 antigen and the capsular polysaccharide. Our objectives are to isolate and characterize these surface antigens while determining their role in pathogenesis. H8, a surface antigen common to all pathogenic <u>Neisseria</u>, has been isolated from a strain of <u>N. gonorrhoeae</u>. Preliminary analyses have substantiated the unusual nature of this protease sensitive antigen. Unlike most outer membrane proteins characterized to date, it lacks aromatic amino acids as well as methionine and cysteine. Significant amounts of two unique fatty acids copurify with the otherwise homogeneous H8 preparation. The H8 antigens of all strains examined to date appear to be antigenically homogeneous, however, they are variably susceptible to proteolysis which suggests some structural variation in the molecule. The polysaccharide capsule has been isolated from two strains of gonococci. There appears to be strain variation with respect to the composition of the neutral sugars. This is reflected in antigenic variation. Although capsular material can be extracted from gonococci irrespective of piliation or opacity phenotype, pilated gonococci, thought to be the virulent phase of the organism, have 1 to 1.5 logs more extractable capsule compared to non-piliated gonococci. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00412-03 LMSF
PERIOD COVERED October 1, 1985, to April 4, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Molecular Analysis of the Lyme Disease Spirochete		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	T. R. Howe	Staff Fellow LMSF, NIAID
Other:	A. G. Barbour	Medical Officer LBP, NIAID
COOPERATING UNITS (if any)		
LAB/BRANCH		
Laboratory of Microbial Structure and Function, Hamilton, MT 59840		
SECTION		
INSTITUTE AND LOCATION		
NIAID, NIH, Bethesda, MD 20205		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.8	0.5	0.3
CHECK APPROPRIATE BOX(ES)		
<input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither		
<input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)		
<p>We have examined the organization of <u>ospA</u> and <u>ospB</u>, genes encoding the two major outer membrane proteins of the Lyme disease spirochete, <u>Borrelia burgdorferi</u>. These genes were each subcloned separately from a recombinant plasmid containing both genes. DNA sequence analysis of portions of these genes and determination of the location and direction of transcription of each <u>osp</u> gene indicates that these genes are co-transcribed. Northern blot analysis of mRNA from <u>B. burgdorferi</u> with DNA probes individually specific for <u>ospA</u> or <u>ospB</u> identified a single, large transcript that hybridized with each probe.</p> <p>This project was terminated April 4, 1986, with the transfer of Dr. Howe to Hybritech Incorporated, San Diego, CA.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE		PROJECT NUMBER
NOTICE OF INTRAMURAL RESEARCH PROJECT		Z01 AI 00413-03 LMSF
PERIOD COVERED		
October 1, 1985, to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)		
Molecular Genetics of <u>Chlamydia trachomatis</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	F. E. Nano	Staff Fellow LMSF, NIAID
Others:	H. D. Caldwell	Research Microbiologist LMSF, NIAID
	N. G. Watkins	Staff Fellow LMSF, NIAID
	T. Hackstadt	Senior Staff Fellow LMSF, NIAID
COOPERATING UNITS (if any)		
Hugh Taylor, Johns Hopkins University School of Medicine, Baltimore, MD		
Helmut Brade, Borstel Institut fur Medizin, Borstel, FRD		
LAB/BRANCH		
Laboratory of Microbial Structure and Function, Hamilton, MT 59840		
SECTION		
INSTITUTE AND LOCATION		
NIAID, NIH, Bethesda, MD 20205		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.55	1.1	0.45
CHECK APPROPRIATE BOX(ES)		
<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)		
<p>A number of recombinant clones expressing surface components of <u>Chlamydia trachomatis</u> and <u>C. psittaci</u> have been characterized during the last year. One set of recombinants, expressing the genus-specific lipopolysaccharide (LPS) of <u>Chlamydia</u>, contains an insert of <u>C. trachomatis</u> serovar L2 DNA. The original recombinant has been extensively manipulated in order to surmise the nature of the recombinant polypeptide product and to facilitate the sub-cloning of the gene into a variety of cloning vectors. Enteric bacteria with well-characterized LPS structures have been transformed with recombinant plasmids harboring the genes which direct the synthesis of the chlamydial LPS-epitope; LPS from these strains have been analyzed structurally to determine the components of the epitope. New recombinant clones from <u>C. trachomatis</u> serovars L2 and B have been isolated that contain genes encoding the major outer membrane protein (MOMP). Expression of the entire MOMP is apparently lethal for <u>Escherichia coli</u> since we are unable to subclone the entire gene into expression vectors. However, expression of MOMP epitopes has been obtained by fusing portions of the MOMP gene to β-galactosidase using lambda gtl1 as a cloning vector. We have found reiterated sequence in or next to the MOMP gene in the B-serovar genome. Another set of recombinants has been identified that expresses a number of surface components of the B-serovar of <u>C. trachomatis</u>, including three recombinants that express putative adhesions proteins of <u>C. trachomatis</u>.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00441-02 LMSF
PERIOD COVERED October 1, 1985, to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Cloning and Expression of Genes of <u>Rickettsia rickettsii</u> in <u>Escherichia coli</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: G. A. McDonald Staff Fellow LMSF, NIAID		
Others: R. L. Anacker Research Microbiologist LMSF, NIAID		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Microbial Structure and Function, Hamilton, MT 59840		
SECTION		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20205		
TOTAL MAN-YEARS: 1.5	PROFESSIONAL: 1.0	OTHER: 0.5
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) From a plasmid (pBR322) clone bank of R strain <u>Rickettsia rickettsii</u> DNA, we have identified an <u>Escherichia coli</u> transformant expressing the 155-Kilodalton (Kd) major protective antigen of <u>R. rickettsii</u> . The transformant, EM24(pGAM21), harbors pBR322 with a 10.1-Kilobase (Kb) insert of rickettsial DNA. A 3.7-Kb PstI fragment containing the antigen-encoding gene was subcloned downstream of the lactose promoter on pUC8 to generate pGAM22. Sonic lysates of <u>E. coli</u> harboring pGAM22 were used to successfully vaccinate mice against a lethal challenge of viable <u>R. rickettsii</u> and to vaccinate guinea pigs protecting them from Rocky Mountain spotted fever upon challenge with <u>R. rickettsii</u> .		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00442-02 LMSF												
PERIOD COVERED October 1, 1985, to September 30, 1986														
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Immunobiology of Guinea Pig Inclusion Conjunctivitis														
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: N. G. Watkins</td> <td style="width: 33%;">Staff Fellow</td> <td style="width: 33%;">LMSF, NIAID</td> </tr> </table>			PI: N. G. Watkins	Staff Fellow	LMSF, NIAID									
PI: N. G. Watkins	Staff Fellow	LMSF, NIAID												
<table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">Others: H. D. Caldwell</td> <td style="width: 33%;">Research Microbiologist</td> <td style="width: 33%;">LMSF, NIAID</td> </tr> <tr> <td>F. E. Nano</td> <td>Staff Fellow</td> <td>LMSF, NIAID</td> </tr> <tr> <td>A. B. Moos</td> <td>Staff Fellow</td> <td>LMSF, NIAID</td> </tr> <tr> <td>W. J. Hadlow</td> <td>Research Veterinarian</td> <td>LPB, NIAID</td> </tr> </table>			Others: H. D. Caldwell	Research Microbiologist	LMSF, NIAID	F. E. Nano	Staff Fellow	LMSF, NIAID	A. B. Moos	Staff Fellow	LMSF, NIAID	W. J. Hadlow	Research Veterinarian	LPB, NIAID
Others: H. D. Caldwell	Research Microbiologist	LMSF, NIAID												
F. E. Nano	Staff Fellow	LMSF, NIAID												
A. B. Moos	Staff Fellow	LMSF, NIAID												
W. J. Hadlow	Research Veterinarian	LPB, NIAID												
COOPERATING UNITS (if any)														
LAB/BRANCH Laboratory of Microbial Structure and Function, Hamilton, MT 59840														
SECTION														
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20205														
TOTAL MAN-YEARS: <div style="text-align: center;">1.45</div>	PROFESSIONAL: <div style="text-align: center;">1.35</div>	OTHER: <div style="text-align: center;">0.1</div>												
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td><input type="checkbox"/> (a) Human subjects</td> <td><input type="checkbox"/> (b) Human tissues</td> <td><input checked="" type="checkbox"/> (c) Neither</td> </tr> <tr> <td><input type="checkbox"/> (a1) Minors</td> <td></td> <td></td> </tr> <tr> <td><input type="checkbox"/> (a2) Interviews</td> <td></td> <td></td> </tr> </table>			<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither	<input type="checkbox"/> (a1) Minors			<input type="checkbox"/> (a2) Interviews					
<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither												
<input type="checkbox"/> (a1) Minors														
<input type="checkbox"/> (a2) Interviews														
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The pathogenesis of recurrent ocular chlamydial infections is thought to be immunologically mediated (i.e., hypersensitivity). However, due to the lack of an appropriate animal model of ocular chlamydial infections, the immunological response to chlamydial antigens during infection and challenge has not been characterized. We have chosen to study the immunopathology of guinea pig inclusion conjunctivitis (GPIC), a <i>Chlamydia psittaci</i> strain which produces both ocular and genital infections in guinea pigs. We have clinically, microbiologically, and histologically characterized both primary GPIC and a delayed hypersensitivity response induced during primary GPIC. The delayed hypersensitivity response is to a heat-labile, genus-specific chlamydial antigen. This ocular response can be induced during chlamydial infections from mucosal sites other than the conjunctiva including genital and intestinal mucosa. The immunodominant antigens during primary infection and following challenge have been identified and recombinant clones expressing these antigens have been isolated. Several purified membrane components from GPIC elementary bodies (EBs) and a clone expressing the genus-specific epitope of lipopolysaccharide have been tested as potential protective antigens. None of the preparations tested have produced protective immunity.</p>														





LABORATORY OF PATHOBIOLOGY
Rocky Mountain Laboratories
Hamilton, Montana
1986 Annual Report
Table of Contents

<u>ZOI AI</u>		
<u>Project Number</u>		<u>Page</u>
Summary		14-1
00061-24	Natural History of Tick-borne Rickettsiae and Their Public Health Significance -- Burgdorfer (Terminated)	14-7
00063-15	Immune Responses to Rickettsial Infections -- Peacock (Terminated)	14-8
00071-15	Studies on Pertussigen -- Munoz	14-9
00082-25	Relation of Viruses to the Genesis of Chronic Disorders -- Hadlow	14-10
00201-07	Structural Characterization of Integrated Viral Genomes -- Garon	14-11
00231-05	Biology of Pathogenic Borreliae and Borrelial Infections -- Barbour	14-12
00268-05	Ecology of Lyme Disease and Related Disorders -- Burgdorfer (Terminated)	14-13
00402-03	Molecular Cloning and Expression of <u>Bordetella pertussis</u> Toxins -- Keith	14-14
00480-01	Pathogen-arthropod Interactions of Vector-borne Diseases Affecting Public Health -- Schwan	14-15

102

103

104

105

106

107

108

109

110

111

112

113

114

115

116

117

118

119

120

121

122

123

124

125

126

127

128

129

130

ANNUAL REPORT
LABORATORY OF PATHOBIOLOGY
ROCKY MOUNTAIN LABORATORIES
HAMILTON, MONTANA
NATIONAL INSTITUTES OF ALLERGY AND INFECTIOUS DISEASES
OCTOBER 1, 1985, TO SEPTEMBER 30, 1986

Members of the Laboratory of Pathobiology bring a broad range of skills and experience to bear in their attempts to define in precise molecular terms those events which are important in host-pathogen relationships. Varied biological systems are carefully dissected using the most modern methods of molecular biology. An important by-product of these studies to define the role of various gene products in the pathogenic mechanism is the potential to produce safe and effective vaccines using pure, well-defined immunogenic products. There is strong interaction among members of the laboratory and with other units, both inside and outside, of the NIH system. The Laboratory of Pathobiology consists of four sections which describe broad areas of research interest. Dr. Claude Garon serves as Acting Chief.

The Molecular Pathobiology Section under the direction of Dr. Jerry Keith has focused on molecular cloning and expression of genes relevant to the toxic components of Bordetella pertussis. Two parallel approaches have been pursued in the identification and isolation of the genes responsible for pertussis toxin. One method utilized a cloned library of pertussis DNA fragments in a direct cloning-expression vector system. Screening was accomplished using polyclonal and monoclonal antibodies to pertussis toxin. In the other more direct approach, fragments of the pertussis genome were screened with a synthetic DNA probe constructed on the basis of available amino acid sequence data. Members of the laboratory have developed about 24 monoclonal antibodies which recognize unfractionated pertussis toxin. Ten of these are specific for three of the five toxin subunits. These monoclonal antibodies have been characterized and recognize the pertussis toxin in both natural and denatured states, making them extremely useful reagents for screening in expression vector systems. In addition, these specific antibodies are being tested to determine their effect on the biofunction of the toxin. In a parallel approach, individual polypeptide subunits from the pertussis toxin have been isolated using high pressure liquid chromatography, amino acid sequences of two specific peptides have been determined and oligonucleotide probes have been synthesized. These probes were used to identify a 4,500 base pair fragment shown to code for all the pertussis toxin subunits. The sequence of the entire 4,500 base pair fragment was determined and the fragment tested in appropriate expression vector systems. The S1 subunit which is known to contain an ADP-ribosylation activity has been successfully expressed in this system (KEITH).

The Arthropod-borne Diseases Section, headed by Dr. Alan Barbour, has concentrated its efforts on two tick-borne spirochetes: Borrelia hermsii, an agent of relapsing fever, and Borrelia burgdorferi, the agent of Lyme disease. Lyme disease is now the most common arthropod-borne disease in the United States. However, in both types of infection, the etiologic spirochetes have developed mechanisms which serve to avoid immune clearance by the host. Studies of relapsing fever have

demonstrated that the spirochetes have abundant surface proteins that differ markedly in their primary structure at various stages of the infection. The Lyme disease borrelia - by an as yet unknown mechanism - survives in the host in spite of a vigorous production of anti-borrelial antibody. Of special interest was the finding that in relapsing fever, the differential expression of the genes encoding these variable antigens appeared related to detectable DNA rearrangements and the duplicative transposition of an antigen-specifying gene from a transcriptionally silent storage site to an active expression site in the chromosome. The recombinational event was non-reciprocal. Further examination of the organization of the genome in the borreliae revealed that certain genes of interest were arrayed on linear plasmids that exist in multiple copies in each spirochetal cell. Some linear plasmids carried the expressed form of the genes, and some plasmids contained the storage loci (BARBOUR).

The Immunopathology Section, with Dr. John Munoz as Acting Head, has continued to characterize the immunopotentiating action of small quantities of crystalline pertussis toxin in a mouse model system. Nanogram doses of pertussigen have been reported to enhance the production of the IgE class of antibodies, to promote the induction of experimental allergic encephalomyelitis and to enhance delayed-type hypersensitivity. Careful analysis of lymphocyte suspensions prepared from various lymph organs and quantitated with specific antibodies to Ig, IgM, Lyt-1, Lyt-2, and Thy 1.2 antigens by means of fluorescence-activated cell sorter techniques has shown marked differences in the percentage of each cell type in normal and pertussigen treated mice. Pertussigen appeared to decrease the percentage of T-lymphocytes (Thy 1.2 containing cells), including helper (Lyt-1+) and suppressor T cells (Lyt-2+). In contrast, a marked increase in the percentage of B cells in the blood (Ig+) and a decrease in these cell types in the spleen and lymph nodes was observed. Similar changes were seen, but to different degrees, in various inbred strains of mice (C57B1/10J, C57, B1/6J, DBA, BALB/c, ASW, SJL, A/WySN). While the effects shown to date have been done with crystalline pertussigen, experiments in progress will attempt to assign these biological effects to specific subunits of the toxin (MUNOZ).

The Pathobiology Section, headed by Dr. Claude Garon, continues to provide research and research collaboration utilizing modern methods of transmission and scanning electron microscopy to define those structural alterations that are related to the pathological condition. While the techniques of ultramicrotomy, nucleic acid microscopy, molecular cloning and other methods allowing a full range of morphological evaluation continues to be exploited, special emphasis has been placed on use of electron immunomicroscopy. Because of the specificity of the reaction between the antigen and its antibody, electron immunomicroscopy offers the greatest potential for the localization and quantification of substances in the cell. While we have successfully identified surface antigens for several years, new techniques involving the use of specialized, low temperature embedding and/or sectioning allow exposure of subsurface components, maintenance of antigenicity and precise ultrastructural localization of important internal antigens in both eukaryotic and prokaryotic cells. Having recently developed procedures for synthesis in the laboratory of uniform, precisely sized, small colloidal gold

markers our laboratory has made a major commitment to the full and effective use of this powerful technology. Given our interest in the structural analysis of retroviral integration and the utility of circular intermediates in molecular cloning, an improved method has recently been developed in this laboratory for the rapid isolation of supercoiled molecules using ion-exchange chromatography of alkali denatured material. Under the conditions employed, contaminating cellular DNA, RNA, and nicked circular molecules are denaturable and are presented to the column as single-strands while intact, covalently closed, supercoiled molecules resist denaturation and are batch eluted from the column as the only remaining duplex species. Small amounts of protein and short, duplex fragments (< 1 kb) are removed in the void volume. There is no need for gradient fractionation. Supercoil fractions are virtually free of contamination with linear molecules as determined by electron microscopy, have an $A_{260\text{ nm}}/A_{280\text{ nm}}$ ratio approaching 2.0, and are suitable for subsequent heteroduplex analysis, molecular cloning, subcloning, or restriction enzyme mapping. The method has been used successfully to purify plasmids up to 45 kb in length and to isolate rare circular replication intermediates from an overwhelming excess of contaminating linear molecules in retrovirus infected cells (GARON).

ADMINISTRATIVE

Dr. Willy Burgdorfer, former Acting Chief of the Epidemiology Branch, retired at the end of 1985. At that time, he became the first Scientist Emeritus of the National Institutes of Health at Rocky Mountain Laboratories. He has remained active during the past year and based on his international reputation and vast experiences in arthropod-borne diseases, remains a valuable resource to our laboratory.

In July 1986 we welcomed Dr. Tom Schwan from the Yale Arbovirus Research Unit to our laboratory as a Staff Fellow in the Arthropod-borne Diseases Section and Dr. Mirosław Szulczynski from the Faculty of Chemistry at Grunwaldzka, Poland as a Visiting Fellow in the Molecular Pathobiology Section.

Guest researchers in the laboratory this past year have included: Dr. Stanley Falkow (Stanford University School of Medicine), Dr. Kenneth Gage (University of Oklahoma) and Kirsten Vadheim (Montana State University).

HONORS AND AWARDS

Dr. Claude Garon

Invited speaker: Montana Society for Medical Technology Annual Convention, Kalispell, Montana, April 10, 1986.

Invited to contribute a chapter on "Electron Microscopy of Nucleic Acids" in Ultrastructure Techniques for Microorganisms, Plenum Publishing Corporation, 1986.

Invited to contribute an original publication to "Gene Analysis Techniques."

Dr. William Hadlow

Invited to review manuscripts for Veterinary Pathology and Journal of Wildlife Diseases.

Invited to present lecture on Slow Viral Diseases and Images of Disease in Ranch Mink at the Olafson Pathology Short Course, Cornell University, Ithaca, NY, August 18-22, 1986.

Member of Advisory Board, Charles Louis Davis DVM Foundation.

Member of Faculty of Discussants, Davis Foundation.

Advisor for Fur Farm Animal Welfare Coalition Ltd.

Dr. Jerry Keith

Invited speaker: AMGEN Corporation, Thousand Oaks, CA, February 20, 1986.

Invited speaker: Smith-Kline-RIT, Brussels, Belgium, June 11, 1986.

Invited lecture: Montana State University, Bozeman, MT, November 1986.

Invited speaker: Pertussis Dinner ASM Meeting, Washington, D.C., March 26, 1986.

Invited speaker: Hamilton Lions Club, Hamilton, MT, July 22, 1986.

Dr. Alan Barbour

Invited speaker: WHO Symposium on the Pathogenesis and Immunology of Treponemal Infection, Los Angeles, CA, December 1985.

Invited speaker: UCLA Symposium on Molecular Strategies of Parasitic Invasion, Park City, UT, January 1986.

Invited speaker: Lyme Disease Symposium, University of Minnesota, June 1986.

Invited speaker: Gordon Conference on Bacterial Toxins and Pathogenesis, New Hampshire, August 1986.

Served as Editor, Lyme Borreliosis Newsletter.

Invited to speak: Medical College of Ohio, December 1985.

Pennsylvania State University, December 1985
SUNY, Stony Brook, March 1986.

Columbia University, College of Physicians and Surgeons, March 1986.

University of California, San Francisco, April 1986.

Stanford University, April 1986.

Invited to review manuscripts for:

Infection and Immunity

Journal of Infectious Diseases

Journal of Clinical Investigation

Journal of American Medical Association

Dr. John Munoz

Reappointed as a Staff Affiliate of the University of Montana

Trustee of the Stella Duncan Memorial Research Fund, University of Montana, Missoula, MT.

Asked to serve as a Judge of the Montana Science Fair.

Asked to write a chapter in a book on "Pathogenesis and Immunity in Pertussis" to be edited by A. C. Wardlow and R. Parton.

Asked to review papers for publication in Clinical Immunology and Immunopathology, Toxicon, Infection and Immunity, and the Proceedings of the National Academy of Sciences of the United States of America.

Asked to give a discussion of B. pertussis and its protective antigens to Ribi Immunochem, Hamilton, MT, June 4, 1986.

Invited to give a seminar at the RML Summer Seminar Session, June 1986.

Will attend the Workshop on Pertussis Vaccine September 22-24, 1986.

Dr. Willy Burgdorfer

The 36th Annual Southwest Conference on Diseases in Nature Transmissible to Man held at Fort Worth, TX. Presented the J. V. Irons Lecture at the Conference's banquet. April 9-10, 1986.

Acarology Summer Program at the Ohio State University, Columbus, OH. Taught a class on Medical-Veterinary Acarology, June 30-July 6, 1986.

Symposium on Lyme Disease sponsored by University of Minnesota, Department of Microbiology and 3-M Company, at Minneapolis, MN. Spoke on "Experimental Infection of Ixodes Ticks with Borrelia burgdorferi", July 27, 1986.

IXth International Congress of Infectious Parasitic Diseases, Munich, Germany. Keynote Speaker - "Ecological and Epidemiological Considera-

tions of the Lyme Disease Spirochete, Borrelia burgdorferi", July 20-26, 1986.

The 41st International Northwestern Conference on Diseases in Nature (INCDNM) Meeting, Salt Lake City, Utah, August 17-20, 1986. Dr. Burgdorfer is Vice-President and will be President next year.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00061-24 LPB
PERIOD COVERED October 1, 1985, to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) (Terminated) Natural History of Tick-borne Rickettsiae and Their Public Health Significance		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI.	Willy Burgdorfer	Research Entomologist (Med) LPB, NIAID
OTHERS:	M. G. Peacock R. A. Anacker	Microbiologist LPB, NIAID Research Microbiologist LMSF, NIAID
COOPERATING UNITS (if any) Univ. Georgia, Athens (C. Greene), Sion, Switzerland (O. Peter), Univ. Ill. (C. J. Holland), Ball State Univ. (R. R. Pinger), Univ. Neuchatel, Switzerland (A. Aeschlimann)		
LAB/BRANCH Laboratory of Pathobiology		
SECTION Arthropod-borne Diseases Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20205		
TOTAL MAN-YEARS: 0.1	PROFESSIONAL: 0.5	OTHER:
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input checked="" type="checkbox"/> (b) Human tissues </div> <div> <input type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Dr. Burgdorfer has retired. This project has been terminated.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00063-15 LPB
PERIOD COVERED October 1, 1985, to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Immune Responses to Rickettsial Infections (Terminated)		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: M. G. Peacock Microbiologist LPB, NIAID		
OTHERS: W. Burgdorfer Research Entomologist (Med) LPB, NIAID D. W. Hackstadt Senior Staff Fellow LMSF, NIAID S. F. Hayes Biol. Lab. Tech. LPB, NIAID		
COOPERATING UNITS (if any) USARMID, Frederick, MD (J. C. Williams), Sion, Switzerland (O. Peter), Orlando Regional Medical Center, Florida (R. G. Brooks)		
LAB/BRANCH Laboratory of Pathobiology		
SECTION Arthropod-borne Diseases Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20205		
TOTAL MAN-YEARS: 0.3	PROFESSIONAL: 0.05	OTHER: 0.25
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) This project has been terminated.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00071-15 LPB
PERIOD COVERED October 1, 1985, to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Studies on Pertussigen		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: J. Munoz Research Microbiologist LPB, NIAID OTHERS: M. Peacock Microbiologist LPB, NIAID		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Pathobiology		
SECTION Immunopathology		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 1.75	PROFESSIONAL: 1.0	OTHER: 0.75
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> Pertussigen (pertussis toxin) (Ptx) is the toxic protein from <u>Bordetella pertussis</u> responsible for most of the known biological activities of pertussis vaccine. We are presently studying the ability of Ptx to induce lymphocytosis in mice in order to find the type(s) of lymphocytes mainly affected by Ptx. To this end, we prepared lymphocyte suspensions from various lymph organs (thymus, lymph nodes, spleen and blood) and determined the percentage of each different type of lymphocyte by means of the fluorescence activated cell sorter (FACS). The fluoresceinated antibodies used to stain each type of lymphocyte were specific to Ig, IgM, Lyt-1, Lyt-2 and Thy 1.2 antigens of these cells. The percentage of each cell type in normal and Ptx-treated mice were determined by the FACS. Ptx decreased the percentage of T-lymphocytes (Thy 1.2 containing cells), including helper (Lyt-1+) and suppressor T-cells (Lyt-2+). In contrast, a marked increase in the percentage of B cells in the blood (Ig+) and a decrease in the percentage of these cells in the spleen and lymph nodes was observed. No changes were noticed in the percentage distribution of T cells in the thymus of Ptx treated mice. Similar changes were seen, to different degrees, in various inbred strains of mice (C57B1/10J, C57 B1/6J DBA, BALB/c, ASW, SJL, A/WysN). Nu/Nu mice (athymic nude mice) also showed a marked leukocytosis after the injection of Ptx with an increase in the percentage of IgM containing cells. Normal Nu/Nu mice contain a small percentage of Thy 1.2 + cells. The percentage of these cells was decreased by treatment with Ptx. Ptx increased markedly the percentage of IgE containing lymphocytes in the spleen and blood (only tissues studied) of many strains of mice, but it was most pronounced in C57B1/10J and C57B1/6J mice. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00082-25 LPB
PERIOD COVERED October 1, 1985, to September 20, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Relation of Viruses to the Genesis of Chronic Disease		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: W. J. Hadlow Research Veterinarian (Pathology) LPB, NIAID		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Pathobiology		
SECTION Pathobiology		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 0.9	PROFESSIONAL: 0.9	OTHER:
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>During the past 20 or so years, this project has been concerned largely with two naturally occurring slow viral diseases of animals, namely, scrapie of sheep and goats and Aleutian disease of mink. Emphasis has been on the host-virus interactions that result in the slow evolution of these diseases--polioencephalopathy in scrapie and progressive glomerulonephritis in Aleutian disease. Quantitative virologic information, obtained by endpoint titration in animal assay systems, and morphologic findings have been used to gain an insight into the pathogenesis of each disease. In scrapie, virus replicates for many months mainly in lymphoreticular tissues before reaching the central nervous system where secondary replication gives rise to fatal degenerative neurologic disease. In Aleutian disease, virus replicates early in lymphoreticular tissues, liver, kidney, and intestine. Depending on the strain of virus and genotype of mink, this soon leads to viremia. When it is sustained, virus in the blood forms soluble complexes with specific antibody that become deposited in glomeruli, giving rise to slowly progressive renal disease. Such information on both diseases has implications for understanding broadly comparable human diseases.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00201-07 LPB
PERIOD COVERED October 1, 1985, to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Structural Characterization of Integrated Viral Genomes		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Claude F. Garon Acting Chief LPB, NIAID OTHERS: Lori L. Jansen Biologist LPB, NIAID		
COOPERATING UNITS (if any) LMM (Martin, Rutledge) LMO/NCI (T. S. Papas)		
LAB/BRANCH Laboratory of Pathobiology		
SECTION Pathobiology		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 1.8	PROFESSIONAL: 0.8	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Retroviruses provide a unique and important system for the study of integrative recombination. Retroviral genomes are integrated with high efficiency through a double stranded, circular intermediate at specific sites within the viral genome, but at a large number of sites in the host chromosome. Often a consequence of this integration event is in addition to viral replication, a change in cell growth characteristics. Modern methods of molecular cloning and analysis allow for the detection and amplification of rare DNA sequences such as a single integrated viral segment. Molecular clones of several newly integrated retroviral genomes have been produced in either plasmid or bacteriophage cloning vehicles using recombinant DNA techniques and have been characterized using electron microscope heteroduplex and R-loop methods. Detection of sequence homology even when rearranged or interrupted by intervening cellular DNA is often accurately mappable in the electron microscope using these methods. These studies have not only shown the arrangement of integrated viral sequences within infected host cell DNA, but have also demonstrated the presence and sequence arrangement of certain viral transforming sequences within normal, uninfected host cells as well. These studies have recently been expanded to include a structural determination of the genetic relatedness of AIDS virus isolates from around the world. Unique inverted repeat sequences structurally resembling transposable elements have been identified in the area of retroviral integrations and molecularly cloned for further analysis. The major objective of these studies has been the application of physical and biochemical techniques to assess the influence of integrative position or flanking cellular sequences on subsequent viral function and to define in molecular terms those events which take place during integrative recombination in eukaryotic cell systems.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00231-05 LPB																								
PERIOD COVERED October 1, 1985, to September 30, 1986																										
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Biology of Pathogenic Borreliae and Borrelial Infections																										
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 40%;">PI: Alan Barbour</td> <td style="width: 40%;">Head, Section</td> <td style="width: 20%;">LPB, NIAID</td> </tr> <tr> <td>OTHERS: Sven Bergström</td> <td>Visiting Fellow</td> <td>LMSF, NIAID</td> </tr> <tr> <td>Willy Burgdorfer</td> <td>Research Entomologist</td> <td>LPB, NIAID</td> </tr> <tr> <td>Claude Garon</td> <td>Acting Lab Chief</td> <td>LPB, NIAID</td> </tr> <tr> <td>Timothy Howe</td> <td>Staff Fellow</td> <td>LMSF, NIAID</td> </tr> <tr> <td>Joseph Meier</td> <td>Graduate Student</td> <td>LPB, NIAID</td> </tr> <tr> <td>Stanley F. Hayes</td> <td>Technician</td> <td>LPB, NIAID</td> </tr> <tr> <td>Tom Schwan</td> <td>Staff Fellow</td> <td>LPB, NIAID</td> </tr> </table>			PI: Alan Barbour	Head, Section	LPB, NIAID	OTHERS: Sven Bergström	Visiting Fellow	LMSF, NIAID	Willy Burgdorfer	Research Entomologist	LPB, NIAID	Claude Garon	Acting Lab Chief	LPB, NIAID	Timothy Howe	Staff Fellow	LMSF, NIAID	Joseph Meier	Graduate Student	LPB, NIAID	Stanley F. Hayes	Technician	LPB, NIAID	Tom Schwan	Staff Fellow	LPB, NIAID
PI: Alan Barbour	Head, Section	LPB, NIAID																								
OTHERS: Sven Bergström	Visiting Fellow	LMSF, NIAID																								
Willy Burgdorfer	Research Entomologist	LPB, NIAID																								
Claude Garon	Acting Lab Chief	LPB, NIAID																								
Timothy Howe	Staff Fellow	LMSF, NIAID																								
Joseph Meier	Graduate Student	LPB, NIAID																								
Stanley F. Hayes	Technician	LPB, NIAID																								
Tom Schwan	Staff Fellow	LPB, NIAID																								
COOPERATING UNITS (if any) State U of NY at Stony Brook (J. Benach), U of CA., Berkeley (R. Lane), Inst of Tech (R. Plasterk, M. Simon), Karolinska Inst, Stockholm (B. Skoldenberg), U of Vienna (G. Stanek), U of Munich (B. Wilske), U of SC (K. Park), CT Agric Sta (L. Magnarelli, J. Anderson), and U of MN (R. Johnson)																										
LAB/BRANCH Laboratory of Pathobiology																										
SECTION Arthropod-borne Diseases Section																										
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20205																										
TOTAL MAN-YEARS: 3.0	PROFESSIONAL: 1.4	OTHER: 1.6																								
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews																										
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Arthropod-borne members of the genus <u>Borrelia</u> cause relapsing fever and Lyme disease. We are taking biochemical and molecular biological approaches to the study of pathogenesis of these human disorders. With regard to the antigenic variation in relapsing fever, we have isolated genes of the proteins that antigenically vary during the course of infection. These genes were found as expression-linked copies and silent copies; the genes were located on linear plasmids. In our studies of Lyme disease, we used a bank of monoclonal antibodies and DNA probes and identified differences between strains. Recombinant DNA clones that express <u>B. burgdorferi</u> surface antigens are candidates for vaccines against Lyme disease.																										

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00268-05 LPB
PERIOD COVERED October 1, 1985, to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Ecology of Lyme disease and related disorders (Terminated)		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: W. Burgdorfer Res. Entomologist (MED) LPB, NIAID		
OTHERS: A. G. Barbour Sr. Staff Fellow LMSF, NIAID S. F. Hayes Biol. Lab. Tech. LPB, NIAID		
COOPERATING UNITS (if any) Univ. Calif, Berkeley (R. S. Lane), Minn. Dept. of Health, Minneapolis (M. T. Osterholm), Univ. Conn. Health Center, Farmington (L. Reik), Munich, W. Germany (K. Weber), Univ. Neuchatel, Switzerland (A. Aeschlimann) Univ. Minnesota (R. C. Johnson)		
LAB/BRANCH Laboratory of Pathobiology		
SECTION Arthropod-borne Diseases Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20205		
TOTAL MAN-YEARS: 0.1	PROFESSIONAL: 0.1	OTHER:
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Dr. Burgdorfer has retired. This project has been terminated.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00402-03 LPB
PERIOD COVERED October 1, 1985, to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Molecular Cloning and Expression of Bordetella pertussis Toxins		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	J. M. Keith	Acting Section Chief LPB, NIAID
Others:	C. Locht	Visiting Fellow LPB, NIAID
	K. S. Marchitto	Sr. Staff Fellow LPB, NIAID
	S. G. Smith	Microbiologist LPB, NIAID
	K. Vadheim	Guest Worker LPB, NIAID
	S. E. Coligan	Senior Scientist LIG, NIAID
	L. S. Mutch	Biologist LPB, NIAID
	M. Szulczynski	Visiting Fellow LPB, NIAID
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Pathobiology		
SECTION Molecular Pathobiology		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 2089205		
TOTAL MAN-YEARS: 4.50	PROFESSIONAL: 3.17	OTHER: 1.33
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Functional objectives are to investigate genetic molecular structure of pathogens, to define the role of gene products in pathogenic mechanisms and to perform studies directed toward development of vaccines using molecular or synthetic production of immunogenic peptides from microbial agents. Our major emphasis is focused on cloning and expression of genes relevant to the toxic components of <u>B. pertussis</u>. Pertussis toxin is the protective component of the whooping cough vaccine; however its toxin activity may cause the harmful side effects associated with current vaccines. The toxin is composed of five dissimilar subunits; subunit S1 contains an ADP-ribosyltransferase activity responsible for most of the toxin's biological activities. The complete structural gene for pertussis toxin has been cloned into <u>E. coli</u> (C. Locht et al., <u>Nucleic Acids Res.</u> (1986) <u>14</u>, 3251-3261) and the nucleotide sequence as well as deduced amino acid sequences of the individual subunits have been determined (C. Locht and J. Keith, <u>Science</u> (1986) <u>232</u>, 1258-1264). Using the sequence data, a Sau3A fragment containing DNA coding for about 75% of the mature S1 subunit was selected to be inserted into the BamHI site of pUC18. Two clones were isolated. Clone 1 contained a Sau3A fragment extending from the second amino acid of the mature protein to amino acid number 187 of S1 in forward orientation (relative to the lac promoter) and clone 2 contains the same fragment in the reverse orientation. Expression of the partial S1 subunit in clone 1 was detected by Western blotting using an anti-S1 monoclonal antibody (Marchitto, K. et al., manuscript in preparation). Expression of S1 in clone 1 was inducible with IPTG. The molecular constructs were made in such a way that the expressed protein lacked the predicted NAD-binding site and therefore lacks toxic activities. This truncated S1 subunit may be useful for development of a safer new generation pertussis vaccine, since S1 is the immunodominant antigen in humans.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00480-01 LPB
PERIOD COVERED October 1, 1985, to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Pathogen-arthropod Interactions of Vector-borne Diseases Affecting Public Health		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Tom G. Schwan Staff Fellow LPB, NIAID OTHERS: Willy Burgdorfer Scientist Emeritus LPB, NIAID		
COOPERATING UNITS (if any) Center of Disease Control, Fort Collins (A. M. Barnes); CA Dept Health Services (B. C. Nelson); Yale University School of Medicine (D. Knudson).		
LAB/BRANCH Laboratory of Pathobiology		
SECTION Arthropod-borne Diseases		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 0.03	PROFESSIONAL: 0.03	OTHER:
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) My primary objective is to develop a research program using molecular approaches to study pathogens and pathogen-arthropod interactions of vector-borne diseases of human importance in the United States. These diseases shall include Lyme disease, Colorado tick fever, plague, murine typhus, and possibly others, which are transmitted to humans by ticks and fleas. Colonies of ticks and fleas known to transmit these infections will be established (if not already present). Recombinant DNA and cloning techniques will be used to develop molecular probes to study pathogenesis of agents within living arthropods and to identify naturally occurring infections in ticks and fleas. Tropisms of pathogens to various organs and tissues in the arthropod will be examined to elucidate vector maintenance and transmission.		

Laboratory of Persistent Viral Diseases
Rocky Mountain Laboratories
Hamilton, Montana
1986 Annual Report
Table of Contents

<u>Z01 AI</u> <u>Project Number</u>		<u>Page</u>
Summary		15-1
00074-14	Genetically Controlled Mechanisms of Recovery from Friend Virus-Induced Leukemia - Chesebro	15-5
00260-05	Role of Endogenous and Recombinant Retroviruses in Leukemia and Differentiation - Chesebro	15-6
00468-01	Biology of Human AIDS Retrovirus - Chesebro	15-7
00072-15	Role of Host and Viral Factors in Resistance to Rabies Virus Infection in Mice - Lodmell	15-8
00262-05	Role of Pentraxins in Acute and Chronic Pathology - Coe	15-9
00085-09	Pathogenesis of Aleutian Disease Virus Infection - Bloom	15-10
00263-05	Structure and Function of the ADV Genome - Bloom	15-11
00086-09	Pathogenesis of Diseases Induced by Non-Oncogenic Retroviruses - Portis	15-12
00264-05	Structure and Function of Endogenous Retroviruses Expressed During Differentiation - Portis	15-13
00199-07	Immunobiology of Aleutian Disease - Race	15-14
00265-05	Immunobiology of Scrapie Virus Infection - Race	15-15
00266-05	Genetic Structure of Murine Retroviruses - Evans	15-16
00386-03	Transformation of Hematopoietic Cells by Avian Tumor Viruses - Palmieri	15-17
00418-03	Immunobiology of Equine Infectious Anemia Virus, a Retrovirus Model for Aids - Chesebro	15-18



Annual Report
Laboratory of Persistent Viral Diseases
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1985 to September 30, 1986

RESEARCH HIGHLIGHTS

Complete cDNA sequence of mouse scrapie-associated prion proteins has been obtained. This sequence shows normal evolutionary divergence from the hamster prion protein sequence indicating that both these genes are probably normal endogenous genes rather than genes of an exogenous infectious agent.

Scrapie agent has been cultivated in vitro in mouse neuroblastoma cells. This should assist in the biochemical characterization of this elusive agent.

New pathogenic effect induced by Friend leukemia helper virus. In addition to the ability of this virus to cause oncogenic transformation of hemopoietic cells, severe hemolytic anemia has now been observed after neonatal virus inoculation.

Recombinant vaccinia virus expressing retroviral envelope proteins was able to induce protective immunity in mice to challenge with Friend murine leukemia virus.

Mouse Rmcf genes responsible for resistance to MCF virus infection in vitro associated with expression of unique MCF retroviral envelope protein detected by monoclonal antibodies. The two known alleles of this gene appeared to encode or regulate expression of two different types of envelope proteins on mouse tail or embryo fibroblasts.

Neurotropic wild mouse ecotropic retrovirus infection was detected in neurons, astrocytes and CNS endothelial cells using specific monoclonal antibodies. Non-neurotropic murine retroviruses appeared to infect endothelial cells but not CNS parenchyma.

New recombinant retrovirus detected in young AKR mice appears to be an intermediate in the generation of recombinant leukemogenic MCF viruses.

Sites of Aleutian disease virus replication in mink infected at birth or as adults were defined using strand-specific nucleic acid probes capable of distinguishing virion and replicative virus forms.

Antigenic variants of equine infectious anemia virus isolated from sequential febrile cycles of an infected horse. Variant-specific antibodies appeared to recognize infected cells in vitro but did not neutralize virus. This suggested that virus neutralization might not be the mechanism of variant selection in vivo.

Transformation-associated properties of avian v-mil-transformed cells
show similarities to cells transformed by the murine v-raf oncogene.

Elevated interferon titers in CNS of rabies-resistant mice may be
mediators of genetically controlled resistance to intraperitoneal rabies
inoculation.

Dissociation and reassociation of subunits of hamster female protein
has been demonstrated. It has been suggested that this could also occur in
vivo and might play a role in the regulation of the function of this protein
in responding to acute inflammation.

Annual Report
Laboratory of Persistent Viral Diseases
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1985 to September 30, 1986

ADMINISTRATIVE REPORT

The following staff changes occurred at LPVD in the past year:
Dr. Soren Alexanderson from Copenhagen, Denmark arrived to work as a Visiting Fellow. Dr. Dan Wiedbrauk, a Staff Fellow, left to take a position in a biotechnology company. Dr. Steven Palmieri, a Senior Staff Fellow, left to take a position in a USDA research laboratory in Georgia.

Dr. Linda Perry, a Guest Worker, is on a sabbatical from the Department of Microbiology, Emory University, Atlanta, Georgia.

Summer student guest workers were: David Lechner, a medical student at the University of Washington, WAMI Program and Dean Dunlap, Montana State University, Bozeman, MT.

Annual Report
Laboratory of Persistent Viral Diseases
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1985 to September 30, 1986

HONORS AND AWARDS

Dr. B. Chesebro

Was presented with the Meritorious Service Medal at Public Health Service Honor Awards Ceremony, Bethesda, MD in May, 1986

Adjunct Professor - Department of Microbiology, Montana State University, Bozeman, MT

Dr. M. E. Bloom

Faculty Affiliate, Department of Microbiology, University of Montana, Missoula, MT

Adjunct Professor of Medical Science, WAMI Program, Montana State University, Bozeman, MT

Dr. J. E. Coe

Faculty Affiliate, Department of Microbiology, University of Montana, Missoula, MT

Dr. D. L. Lodmell

Faculty Affiliate, Department of Microbiology, University of Montana, Missoula, MT

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00074-14 LPVD
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Genetically Controlled Mechanisms of Recovery from Friend Virus-Induced Leukemia		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	B. Chesebro	Chief LPVD, NIAID
Others:	R. Morrison	Staff Fellow LPVD, NIAID
COOPERATING UNITS (if any)		
Drs. B. Moss and P. Earl, LVD, NIAID, Bethesda, MD		
LAB/BRANCH Laboratory of Persistent Viral Diseases, Hamilton, MT 59840		
SECTION		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 2.1	PROFESSIONAL: 1.3	OTHER: 0.8
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The main goal of this project is the elucidation of mechanisms involved in recovery from or persistence of Friend retrovirus complex (FV)-induced erythro-leukemia in mice as a model for human retrovirus diseases such as AIDS and certain leukemias.</p> <p>In continuing studies of the role of the H-2 complex in recovery from FV leukemia, we have found that nonspecific immunosuppression induced by FV is strongly influenced by H-2 genotype. In mapping experiments, the H-2D subregion appeared to be critical in this phenomenon. This same subregion is also most important in spontaneous recovery from FV leukemia. However, spontaneous recovery and resistance to immunosuppression were not always associated, since mice of the H-2D(d/b) genotype failed to recover from leukemia even though they did not appear to be immunosuppressed.</p> <p>In a second phase of this project, we have studied the use of a recombinant vaccinia virus vector expressing the FV envelope gene to induce protection against challenge by FV. Before challenge neutralizing antibodies were usually not detectable, and most challenged mice were successfully infected. However, in vaccinated mice rapid recovery from splenomegaly occurred at about 10 days after challenge. Vaccination appeared to protect by priming of virus-specific T cells which then facilitated a rapid secondary immune response after FV challenge. This secondary response included both neutralizing humoral antibody as well as cytolytic T lymphocytes. The precise role of these effectors in protection is now under investigation.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00260-05 LPVD
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Role of Endogenous and Recombinant Retroviruses in Leukemia and Differentiation		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	B. Chesebro	Chief LPVD, NIAID
Others:	M. Sitbon	Visiting Associate LPVD, NIAID
	L. Evans	Senior Staff Fellow LPVD, NIAID
	C. Garon	Acting Chief LPB, NIAID
COOPERATING UNITS (if any) Dr. B. Sola, Hopital Cochin, Paris, France		
LAB/BRANCH Laboratory of Persistent Viral Diseases, Hamilton, MT 59840		
SECTION		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.6	1.2	0.4
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The main goal of this project is to define different retrovirus-induced pathogenic mechanisms both at the cellular level in intact animals and at the molecular or biochemical level. Retrovirus-induced hemolytic anemia has been found to be an important early pathogenic effect after inoculation of newborn mice with Friend murine leukemia helper virus (F-MuLV). This effect was previously undetected because the main clinical signs of anemia and splenomegaly were identical both in the early hemolytic phase and in the late erythroleukemia phase of the disease. These stages were distinguished in the present work by reticulocyte counts showing high levels during hemolysis and low levels during leukemia. Two closely related F-MuLV strains have been found to differ in their ability to induce both of these pathogenic effects, the lower virulence strain inducing only mild hemolysis and having a very prolonged latency of induction of leukemia. Molecular cloning of this low virulence virus strain has been done in our laboratory and recombinant retroviruses were constructed using the low and high virulence strains. The results indicated that the severity of the early and late pathogenic effects could be dissociated in certain recombinant viruses. This suggested that different portions of the viral genome were responsible for each of these phases of the disease.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00468-01 LPVD
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Biology of Human AIDS Retrovirus		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	B. Chesebro	Chief LPVD, NIAID
Others: None		
COOPERATING UNITS (if any) Dr. T. Folks, LIG, NIAID, Bethesda, MD; Dr. M. Martin, LMM, NIAID, Bethesda, MD		
LAB/BRANCH Laboratory of Persistent Viral Diseases, Hamilton, MT 59840		
SECTION		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 1.0	PROFESSIONAL: 0.2	OTHER: 0.8
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Effort is underway to develop an enumeration assay for infectious AIDS retrovirus. Such an assay would facilitate more accurate and sensitive titration of virus in tissues and body fluids as well as provide a more sensitive means of detecting virus neutralization by antibodies. The assay we are developing would involve detection of foci of infection <i>in vitro</i> in attached cell monolayers by cytopathic effect or focal indirect immunofluorescence using virus-specific antisera. Thus far, we have mutagenized a nonattached cell line which is permissive for AIDS retrovirus and have derived drug resistant and sensitive clones of this cell line. These clones have been fused to attached cell lines and fused progeny have been selected for attachment to plastic and drug resistance. We are now testing fused clones for susceptibility to infection by AIDS retrovirus. This project is also being expanded to attempt to generate AIDS virus-specific monoclonal antibodies which will detect cell surface virus envelope protein and thus be of potential use in a focal immunofluorescence assay for AIDS virus.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00072-15 LPVD
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Role of Host and Viral Factors in Resistance to Rabies Virus Infection in Mice		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: D. L. Lodmell Others: None	Scientist Director	LPVD, NIAID
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Persistent Viral Diseases, Hamilton, MT 59840		
SECTION		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 2.0	PROFESSIONAL: 1.0	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The principal objective of these studies is to determine host and viral factors which influence genetically-controlled murine resistance to, and recovery from street rabies virus (SRV) infections following viral invasion of the central nervous system (CNS). <u>In vitro</u> studies have shown that neither immune serum nor monoclonal antibodies had any effect on intracellular replication or spread of virus from parent to progeny cells during the course of cell division. In contrast, the cell-to-cell spread of SRV, challenge virus standard and ERA rabies viruses from infected to adjacent uninfected cells was inhibited by >99% in both neuronal and non-neuronal cells by antirabies virus immune serum and neutralizing anti-glycoprotein monoclonal antibodies. Non-neutralizing anti-nucleocapsid monoclonal antibodies did not inhibit cell-to-cell viral spread. A better understanding of the factors involved in inhibiting virus spread is essential to our deciphering how resistant strains of mice immunologically or nonspecifically (i.e., interferon) control rabies virus infections following viral invasion of the CNS. Likewise, an investigation of the importance of cellular immunity in the resistance of SJL/J mice to SRV has been initiated with the development of a potential H-2^S target cell that is readily infected with rabies virus. Apathogenic SRV variants have been produced by culturing virus with neutralizing anti-glycoprotein monoclonal antibodies. Virulent and avirulent viruses are being used in comparative pathogenesis studies in A/WySn mice to determine the reasons for their 100% susceptibility to SRV. Continuing pathogenesis studies have shown that high titers of interferon were present in spinal cords of resistant SJL/J mice at approximately the same time the spread of SRV from the spinal cord to the brain ceased. No interferon was detected in cerebrospinal fluids, brains or sera, suggesting that the spinal cord interferon was locally produced. The importance of this interferon as it relates to the failure of SRV to ascend within the CNS of rabies resistant mice is being actively pursued. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00085-09 LPVD
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Pathogenesis of Aleutian Disease Virus Infection		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: M. E. Bloom	Medical Officer	LPVD, NIAID
Other: D. L. Wiedbrauk	Staff Fellow	LPVD, NIAID
R. E. Race	Research Veterinarian	LPVD, NIAID
S. Alexandersen	Visiting Fellow	LPVD, NIAID
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Persistent Viral Diseases, Hamilton, MT 59840		
SECTION		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 3.1	PROFESSIONAL: 2.3	OTHER: 0.8
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The scope of this project is the study of infections of mink with the Aleutian disease of mink parvovirus (ADV). In the past year we developed strand-specific RNA hybridization probes to help localize sites of viral replication. The rationale for the probes is based on the fact that replicative forms of viral DNA (RFs) contain both "+" and "-" sense strands, but that the single stranded virion DNA (SS DNA) is >90% "-" sense; thus, probes "+" in sense react with both the SS DNA and also with the duplex RFs, while "-" sense probes react preferentially with RFs. When tested against DNA extracted from mink infected with virulent ADV-Utah I strain, RFs were detected at 10 days after infection in mesenteric lymph node (MLN), liver, spleen, and gut, but only in gut and MLN at 43 days. SS DNA was noted in these tissues at 10, 43, and 60 days, and in contrast to infected permissive cell cultures, was more abundant than the RFs. These findings suggested that in adult mink, ADV may replicate in gut as well as lymphoreticular tissues. We also used these probes to investigate the recently described fulminant interstitial pneumonitis caused by ADV in newborn mink kits. This disease is characterized by gross pathological lesions confined to the lungs, the development of hyaline membranes, and the presence of viral inclusions. When histological lesions, the presence of intranuclear inclusion bodies, and intranuclear ADV antigen were correlated with levels of viral DNA species, it was concluded that the lung, probably alveolar type-II cells, is the major primary target for viral replication and cytopathology in kits. RFs were also found in low level in MLN, suggesting that ADV replicates in this organ, too, although no cytopathology was evident. The data suggested that the pattern of ADV replication in newborn lung tissue is similar to that seen in permissive cell cultures, but that the replication in other kit organs more closely resembles the pattern described for adult mink. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00263-05 LPVD
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Structure and Function of the ADV Genome		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	M. E. Bloom	Medical Officer LPVD, NIAID
Others: None		
COOPERATING UNITS (if any)		
None		
LAB/BRANCH		
Laboratory of Persistent Viral Diseases, Hamilton, MT 59840		
SECTION		
INSTITUTE AND LOCATION		
NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.9	0.6	0.3
CHECK APPROPRIATE BOX(ES)		
<input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither		
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)		
<p>The purpose of this project is the study of genome structure and function of the Aleutian disease of mink parvovirus (ADV). Approximately 85% of the DNA sequence of the attenuated ADV-G strain has been deduced using the M13-based dideoxy method. The findings suggest that the overall organization is similar to that of other nondefective parvoviruses, but that homology at both the nucleotide and predicted protein level is <50%. Molecular clones representing 15-88 map units (MU) were derived directly from two <u>in vivo</u> passaged virulent ADV strains and compared to ADV-G. Detailed restriction mapping indicated that the viruses were very closely related and that the segments of all three viruses were the same size. Clones of all three expressed antigens in <u>E. coli</u> that reacted with sera specific for virion, capsid proteins, but, in spite of the fact that the DNA segments are identical in length, the proteins encoded by clones of the two <u>in vivo</u> viruses were 2-3 kd larger than those of ADV-G. This finding may be important because the capsid proteins found in particles from virulent viruses also are 2-3 kd larger than the comparable ADV-G capsid proteins. DNA sequence comparison between these two viruses and ADV-G is underway.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 AI 00086-09 LPVD
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Pathogenesis of Diseases Induced by Non-Oncogenic Retroviruses		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	J. L. Portis	Medical Officer LPVD, NIAID
Others: None		
COOPERATING UNITS (if any) Dr. W. G. Stroop and Dr. J. J. Townsend, Neurovirology Research Laboratory, Univ. Utah School of Medicine, Salt Lake City UT; Dr. M. B. A. Oldstone, Scripps Clinic and Research Foundation, La Jolla, CA		
LAB/BRANCH Laboratory of Persistent Viral Diseases, Hamilton, MT 59840		
SECTION		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
1.7	0.7	1.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.) <p>It has recently been recognized that some human retroviruses are capable of replicating in the central nervous system, and it is estimated that up to 80% of patients with diagnoses AIDS exhibit manifestations of CNS pathology. The pathogenesis of CNS diseases induced by retroviruses is not well understood. We are studying a murine retrovirus (WM-E) which was originally isolated from wild mice and has the capacity to cause a paralytic disease in some strains of laboratory mice with a latency of 12-20 weeks. The primary goals of this project are to identify the cells in the CNS in which this virus replicates and the mechanisms by which it causes motor neuron pathology. Using monoclonal antibodies specific for the gp70 of WM-E and nonreactive with that of endogenous viruses, we have localized sites of replication to spinal cord and brain stem. Viral antigen has been detected in endothelial cells and astrocytes as well as neurons. Murine retroviruses which do not cause neurological disease also infect the CNS. However, viral replication has only been detected in endothelial cells with no evidence of virus spread to the parenchyma. Thus, the capacity of WM-E to produce paralytic disease appears related to its capacity to spread from endothelial cells in the CNS to the parenchyma. In addition to the CNS, WM-E was also found to infect organs of external secretion. High titers of infectious virus were found in saliva, semen and uterine secretions. Horizontal transmission of this virus between adult mice occurred with high frequency, viremic males being the sole source of infection. Infectious virus was transferred to the female reproductive tract during copulation and was found within the oviducts associated with motile spermatozoa. Though saliva contained high concentrations of virus, oral transmission did not occur. The high efficiency of sexual transmission was associated with a unique tropism of WM-E for epithelial cells lining the epididymis. F-MuLV which was transmitted horizontally, but with low efficiency, did not replicate in these cells.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00264-05 LPVD
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Structure and Function of Endogenous Retroviruses Expressed During Differentiation		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	J. L. Portis	Medical Officer LPVD, NIAID
Others	R. Buller	Staff Fellow LPVD, NIAID
	M. Sitbon	Visiting Fellow LPVD, NIAID
	B. Chesebro	Chief LPVD, NIAID
COOPERATING UNITS (if any) Dr. Aftab Ahmed, Emory University, Atlanta, GA; Dr. Linda Perry, Emory University, Atlanta, GA		
LAB/BRANCH Laboratory of Persistent Viral Diseases, Hamilton, MT 59840		
SECTION		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	1.3	0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The goal of this project is to identify murine retroviral gene products that are expressed during development and their possible role in resistance to retrovirus-induced diseases. Using a panel of monoclonal antibodies derived from mice undergoing graft-versus-host disease, we have identified a group of endogenous gp70 molecules expressed both in embryonic and adult tissues. One of these molecules is expressed by secondary embryo cultures derived from mice expressing the resistance allele of the Rmcf locus, a gene which restricts the replication of recombinant MCF viruses <u>in vitro</u>. This gp70 exhibits an epitope unique to gp70 of MCF viruses. A second gp70 molecule which is expressed by the same cells derived from Rmcf^S mice resembles serologically that of xenotropic viruses. We have now carried out backcross analysis of progeny from (Rmcf^R X Rmcf^S) X Rmcf^S mice and found that restriction of MCF virus replication (Rmcf^R) segregated with the endogenous MCF but not the xenotropic gp70. The genes encoding these two gp70 molecules are allelic and their expression appears to be coordinately regulated during embryonic development. Expression has been detected in a subpopulation of cells of the erythroid lineage in fetal liver. In the adult these proteins are virtually undetectable unless erythropoiesis is stimulated. Lymphoid cells in adult hematopoietic organs express a third gp70 which is related to that of xenotropic viruses but is serologically distinguishable from the xenotropic gp70 found on embryonic cells. Since we now have antibodies which distinguish the two alleles of the Rmcf gene, we are carrying out backcross analyses to determine whether resistance to retrovirus induced erythroleukemia segregates with one of the endogenous gp70 alleles. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00199-07 LPVD
PERIOD COVERED October 1, 1985, to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Immunobiology of Aleutian Disease		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	R. E. Race	Research Veterinarian LPVD, NIAID
Others:	M. E. Bloom	Medical Officer LPVD, NIAID
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Persistent Viral Diseases, Hamilton, MT 59840		
SECTION		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 0.6	PROFESSIONAL: 0.3	OTHER: 0.3
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) <p> Monoclonal antibodies were used to study antigenic differences among strains of ADV and to characterize viral proteins <u>in vitro</u> and <u>in vivo</u>. Highly virulent Utah I ADV was clearly delineated from the tissue culture-adapted avirulent ADV-G strain. This specificity could be demonstrated by indirect immunofluorescence (IFA) against infected cultures of Crandell feline kidney cells or against tissues of Utah I ADV-infected mink. Immunoprecipitation analyses utilizing various mAbs identified specific antigenic determinants. When immunoprecipitation-defined reactivities were correlated with IFA tissue and <u>in vitro</u> patterns of reactivity it was apparent that the virus-associated antigenic determinants recognized <u>in vivo</u> were proteolytic products of viral structural proteins. Thus, intact structural ADV proteins were not identified <u>in vivo</u>. However, structural proteins were detected <u>in vitro</u> when ADV-G or Utah I ADV-infected CRFK cells were analyzed. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00265-05 LPVD
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Immunobiology of Scrapie Virus Infection		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	R. E. Race B. Chesebro J. Keith C. Locht	Research Veterinarian Chief Senior Staff Fellow Visiting Fellow LPVD, NIAID LPVD, NIAID LPB, NIAID LPB, NIAID
COOPERATING UNITS (if any) Dr. A. Haase, Chief, Dept. Microbiology, University of Minnesota, Minneapolis, MN		
LAB/BRANCH Laboratory of Persistent Viral Diseases, Hamilton, MT 59840		
SECTION		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 1.6	PROFESSIONAL: 0.9	OTHER: 0.7
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Scrapie is a naturally occurring spongiform encephalopathy of sheep and goats which causes clinical and pathological changes similar to those of Creutzfeld-Jakob and Kuru diseases of man. A unique protein called prion protein (PrP) has been found to be a major component of purified samples of scrapie infectivity and is believed by some people to be the actual infectious agent. We previously isolated two cDNA clones of the PrP mRNA from scrapie-infected mouse brain. In the past year, we have finished determining the complete sequence of these mouse prion protein cDNAs. Comparison of the sequence with that of hamster PrP indicated that these very homologous genes showed nearly equivalent evolutionary divergence in both their coding and noncoding regions which confirmed previous findings that the PrP genes are normal endogenous genes of many species rather than genes of an exogenous infectious agent. The possibility that post-translational modification of the endogenous gene product results in creation of the infectious scrapie agent has not been completely excluded.</p> <p>Although several attempts have been made to adapt the scrapie agent to <u>in vitro</u> growth, the few positive cultures reported have been of low titer. Nevertheless, adaptation of the agent to cell culture could permit detailed characterization of the agent and offer the possibility of <u>in vitro</u> titrations and a marked reduction in bioassay related problems. Toward this goal neuroblastoma cell lines of mouse origin were successfully infected with scrapie agent. Infected cultures were maintained through 21 passages, well beyond the number needed to assure that replication had occurred. Infection appeared to be species-specific in that agent derived from hamster brain did not infect the mouse derived neuroblastoma cell lines. Cells from currently infected cultures were cloned in hopes of increasing infectivity levels and additional cell lines are being analyzed in anticipation that others even more receptive to scrapie might be identified.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00266-05 LPVD
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Genetic Structure of Murine Retroviruses		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	L. H. Evans	Senior Staff Fellow LPVD, NIAID
Others:	J. D. Morrey	Staff Fellow LPVD, NIAID
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Persistent Viral Diseases, Hamilton, MT 59840		
SECTION		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 3.0	PROFESSIONAL: 2.0	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Retroviruses commonly undergo genetic alterations. These include point mutations, which have been documented with the lenti viruses such as visna, EIAV and the AIDS virus, as well as major substitutions with host genes which have been documented with murine leukemia viruses (MuLVs). Ecotropic MuLVs (MuLVs which infect only murine cells) undergo recombination with endogenous sequences of the mouse to generate polytropic viruses (MuLVs which infect cells of other species). The major goal of this project is to characterize recombinant MuLVs generated between ecotropic MuLVs and gene sequences of the mouse, and to define their role in the disease process. Initial studies demonstrated that polytropic MuLVs isolated after inoculation of an erythro leukemia virus, Friend (F) MuLV, were derived from different endogenous sequences than those isolated after inoculation of a lymphocytic leukemia virus, Moloney (M) MuLV. This finding has been confirmed using an immunofluorescence assay to analyze the frequency of recombinant MuLV types present in large virus populations from infected mice. Further studies have indicated that pseudotyping of the ecotropic virus by envelope proteins of polytropic viruses correlated with the development of lymphocytic leukemia. Pseudotyping yields ecotropic virions with an altered range of infectivity and may facilitate the infection of cells which ultimately become transformed. Ecotropic pseudotyping may reflect the generation of specific types of polytropic viruses.</p> <p>AKR mice harbor an endogenous ecotropic virus which recombines with endogenous nonectropic sequences to generate oncogenic polytropic viruses. These recombinant viruses possess two nonectropic sequences; one corresponding to the 5' end of the envelope gene (<u>env</u>), and another which includes the long terminal repeat (LTR). The nonectropic sequences are thought to be derived from two different retroviral species. Intermediates have been described which contain the LTR sequence, however the source of the 5' <u>env</u> sequence was not known. We have identified an intermediate containing the 5' <u>env</u> sequence in young AKR mice.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00386-03 LPVD
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Transformation of Hematopoietic Cells by Avian Tumor Viruses		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	S. Palmieri	Senior Staff Fellow LPVD, NIAID
Others: None		
COOPERATING UNITS (if any)		
None		
LAB/BRANCH		
Laboratory of Persistent Viral Diseases, Hamilton, MT 59840		
SECTION		
INSTITUTE AND LOCATION		
NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5
CHECK APPROPRIATE BOX(ES)		
<input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither		
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)		
<p>An avian retrovirus containing only the <u>v-mil</u> oncogene was analyzed for its ability to induce a transformed phenotype in chicken embryo fibroblasts. Infected cultures exhibited an altered morphology, disarranged actin cable filaments, and a decrease in the amount of cell-surface fibronectin. In addition, these cells showed a high level of plasminogen activator protease activity and were also capable of growth under low serum conditions. In contrast, only small numbers of foci under agar and colonies in semisolid medium were induced by this virus relative to Mill Hill 2 and Rous sarcoma viruses. Forty percent of the birds inoculated in the wing web with <u>v-mil</u>-infected cells developed slow growing tumors at the site of injection; no evidence of leukemia was detected. Our results indicate that the <u>v-mil</u> oncogene is transforming both <u>in vitro</u> and <u>in vivo</u>, that <u>v-mil</u> is functionally related to its homologous murine counterpart <u>v-raf</u>, and that the <u>v-mil</u> and <u>v-myc</u> oncogenes in MH2 can independently transform fibroblasts.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00418-03 LPVD
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Immunobiology of Equine Infectious Anemia Virus, a Retrovirus Model for AIDS		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	B. Chesebro	Chief LPVD, NIAID
Others:	S. Carpenter	Staff Fellow LPVD, NIAID
COOPERATING UNITS (if any) Dr. M. Sevoian, Univ. Massachusetts, Amherst, MA		
LAB/BRANCH Laboratory of Persistent Viral Diseases, Hamilton, MT 59840		
SECTION		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 1.1	PROFESSIONAL: 1.1	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Antigenic variation has been suggested as a means by which several lentiviruses, including equine infectious anemia virus (EIAV), AIDS retrovirus and visna virus, are able to persist in spite of a virus-specific host immune response. The goal of this project is to elucidate the factors important in the generation and selection of genetic and antigenic variants of EIAV with regard to the role of variation in viral persistence and the pathogenesis of disease. We recovered isolates of EIAV from two early cycles of clinical disease in an experimentally infected horse. These isolates were found to be genetic variants as determined by RNase T₁ resistant oligonucleotide fingerprint analysis. Furthermore, the isolates could be antigenically distinguished by antibody which bound to the surface of virus-infected cells, supporting the concept of a sequential development of antibody which recognizes emerging viral variants. However; no neutralizing antibody was detected during the early, febrile periods from which these variants were isolated. Instead, neutralizing antibody was detected later in the course of disease, but neutralizing antibody specific for the later appearing virus variant was detected prior to neutralizing antibody specific for the earlier appearing variant. These results suggested that neutralization might not be the mechanism for selection of EIAV variants. However, non-neutralizing antiviral antibody might still select for variants through recognition and elimination of certain virus-infected cells. Alternatively, the antibody specificity may be a response to, rather than a cause of, viral variation <u>in vivo</u>. In an attempt to more clearly define the role of antibody in the selection of viral variants, we plan to analyze the genetic and antigenic relatedness of six isolates of EIAV which we recovered from consecutive febrile periods of an experimentally infected horse.</p>		



ROCKY MOUNTAIN OPERATIONS BRANCH
Rocky Mountain Laboratories
Hamilton, Montana
1986 Annual Report
Table of Contents

Introduction	16-1
General Overview	16-2



Annual Report
Operations Branch
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1985, to September 30, 1986

Introduction

The branch provides all services necessary to the professional staff in the pursuit of their investigations. Fiscal support includes budget management, procurement and initiating payments and follow up on financial obligations related to purchases, contracts, staff and official guest travel and expenses for conferences held at RML. Other support covers the following areas: personnel, communications, library services, secretary backup service, grounds care, custodial, security, media preparation, waste disposal including hazardous wastes and radioactive wastes, glassware cleaning, photography, animal rearing and care, motor pool, operation of power plant and full maintenance and minor laboratory renovations in every area except electronics.

Last October the IRP, NIAID decided not to utilize the Jefferson School as a conference center and initiated action to cancel the 10-year lease which had been previously negotiated between the Hamilton School Board and the government. The termination of the lease became effective on 30 June 1986.

In March the new incinerator facility which had been under construction since late summer of 1985 was completed and came on line. This unit replaced a system that had been seriously deteriorating for about three years and employs state-of-the-art technology to essentially reduce air pollution to zero. It is also equipped with a secondary incinerator that can be fired up in case the primary unit has to be taken out of service for repairs.

HD-3, the building which was originally renovated to house primates, was put into use in June to house mice and hamsters that are used by Dr. Race in scrapie research. It is equipped with a HVAC system, preparatory lab, wash room and three animal rooms. One animal caretaker takes care of 5000-6000 animals in this facility.

In June and July the library moved all of its journal collection and part of the reference collection up through the year 1971 (7000 volumes) into the recently renovated area on the third floor of building 2. This move provided space in the main library area for, hopefully, another six years accumulation of journals.

General Overview of the Responsibilities of Operations Branch

The fiscal and procurement department manages a budget of over \$1,800,000. Payroll is not included in this figure. It covers only the purchase of supplies and equipment used in the operation of the laboratories. Timekeeping and submission of the payroll are also handled in this unit.

Personnel handles all actions and advises on personnel matters. This department is also charged with operation of the Job Training Partnership Act in association with the local Montana State Employment Office. However, because of budget constraints to the state on this program, it was only active for a short time this past year. Also handled by Personnel are persons under the following programs: Stay-in-School, Summer Aides, Work Study, Student Volunteers, Visiting Program, and students studying for advanced degrees.

Most of the biological media used in the research laboratories is prepared in a special laboratory by a technician. Glassware is cleaned and sterilized in the glassware department for reuse in the laboratories.

The Graphic Arts Department provides full professional services necessary in the laboratories with the exception of medical artistry.

The library provides a full range of services for the RML staff, i.e., selections and acquisitions, cataloging, circulation, inter-library loans, reference and bibliographic services, computerized data base searches, and preparing periodicals for binding.

The Animal Unit raises guinea pigs, 16 strains of mice, 7 strains of hamsters, and a colony of microtus. They breed and raise approximately 86,000 animals a year. An additional 2,000 animals are purchased annually from outside sources, including mink, rabbits, mice, chickens and hamsters. After rearing, care is provided for these animals while they are under experiment.

The Chief of the Branch is responsible for labor management work and administering the technical aspects of the A-76 contracts for Security, Custodial and Operation of the Power Plant with the respective private contractors. Security is provided by a guard on duty every night and all day on weekends and holidays. Custodial services are provided in five laboratory buildings daily except weekends and holidays. Power plant operation provides heat, steam, compressed air, vacuum and emergency power to the entire laboratory complex.

The maintenance department provides repair, service and renovation work in plumbing, electrical, sheet metal, carpentry, air conditioning and refrigeration, including ultra low temperature boxes. With the exception of electronic work, all maintenance is done by the staff. Also provided are demineralized and distilled water. A motor pool consisting of 10 vehicles is maintained. Grounds care including snow removal is provided.



NIH Library Building
National Institutes of Health
Bethesda, Md. 20892



<http://nihlibrary.nih.gov>

10 Center Drive
Bethesda, MD 20892-1150
301-496-1080



